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References:

Raman, C. V. (1949) The theory of the Christiansen experiment. *Proc. Indian Acad. Sci.*, A, **29**: 381-90.

Sahni, B. (1936a) Wegener's theory of continental drift in the light of Palaeobotanical evidence. *J. Indian bot. Soc.*, **15**: 31-32.

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Some Observations on Blossom Biology and Fruit Formation in *Hibiscus esculentus*

BY

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(Accepted for publication, 20th February, 1953)

ABSTRACT

The data presented herein on certain floral characters and development of fruit in six varieties of the "bhendi", *Hibiscus esculentus* L., reveal that: (i) the flower, in general, takes 20-22 days to reach the maximum size from the time of the initiation of the bud, (ii) the size of the flower bud a day prior to anthesis appears to be a varietal character, (iii) the anthers dehisce soon after the unfurling of the twisted corolla and the stigma remains receptive as long as the flower is open, (iv) under field conditions, cross-pollination is found to be the general feature, while self-pollination is by no means totally absent, (v) natural cross-fertilisation takes place to an appreciable extent, especially when two or more varieties are closely planted, (vi) the pollen grains lose their viability considerably even within 24 hours of the dehiscence of the anthers when stored at the room temperature (30°C.), but that they remain viable up to about 144 hours when stored over $CaCl_2$ at O° C., (vii) in the variety "P. 13", the fruit attains its maximum length by about the tenth day after pollination, i.e., long before the maximum weight is reached, the fruit being in a fit condition for harvest as vegetable by the seventh day, and that (viii) unequal deposition of pollen on stigmatic lobes may result in the curling of fruit in varieties which normally produce straight fruits.

INTRODUCTION

A proper understanding of the floral characters and the mode of pollination of a crop is a basic necessity for any work on crop improvement by pure line selection or hybridisation. Likewise, knowledge of the development of the fruit, especially in vegetable crops in which the tender fruit is the valued product, would be of practical interest. Information available on these aspects in the case of the "bhendi", *Hibiscus esculentus* L., being limited, some investigations on the floral characters, pollination, pollen storage and viability, and fruit formation were undertaken during

the years 1945 to 1947 as a prelude to a study of the practical utilisation of hybrid vigour in this vegetable. Certain interesting features observed during the course of the study are presented in this communication.

Materials and Methods

The materials employed in the various experiments were obtained from one or all of the following six varieties: "P. 13", "Pal", "Podugu", "Mullu", "Long Green W.V." and "Long Green R_8P_5", which have been briefly described elsewhere (Venkataramani, 1952).

The cultivation and maintenance of the crop throughout the course of the study conformed to recognised cultural practices in vogue in South India. For the sake of convenience and conciseness, the methods adopted in the different experiments are described under appropriate heads in the following part.

Experimental Observations and Results

1. *The flower—A general description*:

The flowers are solitary and axillary and are produced in acropetal succession. The bracts are two in number and linear; the bracteoles are ten or more forming a whorl of epicalyx. The calyx is tubular and indistinctly five-lobed. The corolla consists of five petals which are free except at the very base where they are fused with the staminal tube. The stamens are monadelphous, the staminal tube enclosing the slender style. The numerous anthers are reniform, one-celled and are borne on short filaments. The pollen grains are large and spherical, about 160 μ in diameter (excluding the spines), pale yellow in colour; exine thick and granular, provided with numerous spines which are long and conical with broad bases and blunt tips and evenly distributed over the surface of the grain. The pistil consists of an ovary and a slender stylar column terminating in short branches bearing the stigmatic lobes which are usually five, but sometimes more depending on the number of the locules of the ovary.

As in the case of the vegetative parts, differences are also observed in the colouration of the floral parts such as the pedicel, calyx and corolla. The basic or ground colour of the petal in all the varieties is distinct yellow (sulphur yellow). In some varieties, the petal spot is found only on the inner surface of the petal, while in others it is met with on both surfaces. In some, the deep

red colour of the spot extends to the veins of the petal (Venkataramani, 1952).

Abnormalities in the floral structures are occasionally met with and a few instances noticed by the author have been already described and compared with other earlier reports (Venkataramani, 1948).

2. *The development of the flower :*

The rudimentary flower bud was seen as a distinct speck in the axil of the leaf. The epicalyx at this stage was fairly well marked out and easily discernible. Such of those buds, four in each of ten plants of six varieties, were tagged with small paper labels with the date on them and daily inspection of the plants enabled observation of the development (increase in size) of the flower buds. The growth was very gradual as represented in Plate I, Fig. 1. The number of flowers blossoming at different time intervals in six varieties is presented in Table I.

TABLE I

NUMBER OF FLOWERS BLOSSOMING AT DIFFERENT TIME INTERVALS

Varieties	No. of flowers blossomed after							
	18	19	20	21	22	23	24	25 days
P. 13 ..	—	2	28	7	3	—	—	—
Pal ..	—	1	—	25	12	2	—	—
Podugu ..	—	—	20	—	18	2	—	—
Long Green W.V. ..	—	4	17	19	—	—	—	—
Mullu ..	—	—	—	16	20	2	2	—
Long Green R_8P_5 ..	—	—	9	8	23	—	—	—

The mean interval between the first appearance of the bud and flowering varied from 20-22 days in the different varieties.

3. *Size of flower bud on the day previous to flowering*:

The length of the bud (excluding the pedicel) was measured as this was found to be a satisfactory indicator of bud size. Daily inspection of the plants enabled easy observation and the bud size prior to flowering was determined in six varieties, in each case 50 counts being taken. The results are summarised in Table II.

TABLE II

SIZE OF THE FLOWER BUD (in cm.) IN SIX VARIETIES ON THE DAY PRIOR TO ANTHESIS

Varieties		Range	Average
P. 13	..	2·8 to 3·1	3·0
Pal	..	2·4 — 2·9	2·7
Podugu	..	3·0 — 3·4	3·3
Long Green W.V.	..	4·1 — 5·2	4·8
Mullu	..	2·7 — 3·2	3·0
Long Green R_8P_5	..	3·4 — 3·7	3·6

The size of the flower bud in the six varieties ranged from 2·4 to 5·2 cm., the average size being 2·7 to 4.8 cm. The buds of the variety "Pal" were the smallest, while those of the "Long Green W.V." were the largest. A slight difference from variety to variety was also discerned (Plate I, Fig. 2).

4. *Anthesis :*

The first symptom of the unfolding of the flower bud was marked by the appearance of a slit in the calyx (Text-fig. 2). The calyx split opened on one side by 10-30 — 11 p.m. and the corolla was seen increasing in size. The slit in the calyx gradually increased (Teyt-figs. 3 and 4). By about 4-30 a.m. the corolla was seen emerging out of the slit in the calyx lobe (Text-fig. 5) and in another 30 minutes a small opening was seen at the top of the twisted corolla and the flower was almost fully blossomed by about 6 a.m. (Text-figs. 6 and 7). The anthers did not dehisce when

TEXT-FIGURES 1-12

TEXT-FIGS. 1-9: Diagrammatic representation of the opening and withering of the flower in *Hibiscus esculentus*. Note the small slit in the calyx lobe (TEXT-FIG. 2), its gradual increase in size (TEXT-FIGS. 3 and 4), protrusion of the twisted mass of corolla out of the split calyx (TEXT-FIG. 5), the small opening at the top of the unfurling petals (TEXT-FIG. 6), a fully blossomed flower (TEXT-FIG 7), and the withering and twisting of the petals (TEXT-FIGS. 8 and 9). TEXT-FIGS. 10-12: Curved and normal fruits of the variety "P. 13". Note the normal development of seed only in one locule of the curved fruit (TEXT-FIG. 11).

the bud was intact and dehiscence of anthers occurred only after the opening of the corolla. A few anthers at the top dehisced first, but within a short time all the anthers gradually burst, the pollen grains appearing in clusters over the anther lobes. The pollen being viscid, the dispersion of the grains was effected mainly by external agents such as insects. By about noon the petals began to wither and they were completely twisted in the evening (Text-figs. 8 and 9).

5. *Pollination :*

Under natural conditions cross-pollination was the general feature, failing which self-pollination took place by the twisting of the corolla and the mechanical deposition of the pollen on the stigmatic lobes.

6. *Receptivity of the stigma :*

The duration of the receptivity of the stigma was determined by pollinating emasculated and bagged flowers with viable pollen at different time intervals.

The stigma was receptive 12 hours prior to anthesis and 90% fruit set was obtained. It remained so till about 4 p.m. on the day of anthesis. Pollinating the stigma at 1 p.m. the next day gave only 45% fruit set and thereafter no fruit set was secured.

7. *Extent of natural cross-fertilisation :*

Two varieties with contrasting characters, viz., (i) "Long Green W. V." with all plant parts green in colour and the leaf lobing being shallow and (ii) "P. 13" with the stem green-red in colour and the leaf deeply lobed, were employed. The green colour of the stem was recessive and so was the shallow lobing character of the leaf. The resulting hybrid between these two resembled the variety "P. 13", the plant with all dominant characters. A small plot consisting of 22 rows was chosen, the distance between each row being two feet. Seven plants were grown in each row. The first four rows were planted with the variety "P. 13" and the remaining ones with the other variety. All plants of "P. 13" were in flower when the variety "Long Green W.V." began to flower. The fruits of the latter variety were collected separately to determine the extent of crossing taking place at different distances from the nearest "P. 13" plant.

Natural cross-fertilisation did occur and its extent ranged from 4·0 to 31·7 per cent., depending upon the distance between the two parental plants. Even at a distance of 34 feet as high a percentage of crossing as eight was recorded.

8. *Effect of mixtures of pollen on fruit set :*

To study the effect of a mixture of pollen from two different varieties on fruit set, an experiment was planned with three main treatments in view, viz., (A) viable pollen of two varieties was mixed and applied to the stigma, (B) the stigma was first pollinated with foreign pollen and then with self pollen at intervals of half an hour up to three hours, and (C) the stigma was pollinated with self pollen first and with the foreign pollen as in treatment B. The flowers (emasculated) thus treated were bagged and the number of fruit set was recorded. The fruits were later harvested separately and seeds collected from each of them were sown in separate seed-pans to study the nature of the seedlings produced. In this experiment also, as in the case of the previous one, the two varieties, "Long Green W.V." and "P. 13", showing contrasting characters were employed.

The following indications were obtained: (i) treatment A, out of 20 fruits seven produced hybrid seeds and the remaining ones seeds of both pure and mixed origin, (ii) treatment B showed that none of the fruits produced pure seed; in the ½ to 1½ hour treatments some fruits (26·3%, 10% and 10%) gave pure seeds mixed with hybrid ones, while those from the 2 to 3 hour treatments produced only hybrid ,seed, and (iii) treatment C showed that in the ½ to 1½ hour treatments the majority of the fruits produced seed of pure origin with a few (20%, 11·1% and 15%) producing a mixture of pure and hybrid seed, while in the 2 to 3 hour treatments all the fruits gave pure line seed.

9. *Longevity of the pollen :*

Preliminary trials indicated the futility of artificial germination of pollen grains on slides with water or sugar solutions, and great control of the medium employed was necessary to secure satisfactory germination. Germination of the pollen grains on the stigmatic lobes was successful and the germinated grains could be easily traced in the stigmatic tissue. The method adopted for this test was briefly as follows: the stigmatic lobes were separated and thin longitudinal sections were taken with a safety razor blade.

Each such section was placed on a microscope slide and a known number of pollen grains was dusted on the stigmatic surface. To prevent drying out, the slides were kept in a moist chamber and the sections were viewed under a microscope to spot out the germinating grains after 10-15 minutes. With thin sections it was possible to locate the pollen-tubes in the interstices of the hairy outgrowth or papillae of the stigma. Staining with cotton blue in lactophenol was advantageous and by spreading out the tissues a little bit by applying a gentle pressure to the cover glass the stained pollen grains and tubes could be clearly seen (Plate I, Figs. 3 and 4).

To determine the longevity of the pollen, the pollen grains were collected from unburst anthers the evening previous to flowering and also as soon as the anthers dehisced on the day of anthesis. The germination capacity of the pollen was determined by testing as described above soon after collection and at intervals of two hours from 6 a.m. to 8 p.m. on the day of flowering. The pollen was viable 12 hours previous to anthesis and it lost its viability to a considerable extent by the evening of the first day of flowering. It, therefore, appeared necessary to keep the pollen stored under certain conditions favourable for the retention of viability, should it be required for use at different intervals.

Pollen grains were collected from dehisced anthers soon after anthesis, kept in gelatin capsules, and stored as follows:

(i) at room temperature (30°C.)
- $-CaCl_2$
- $+CaCl_2$, i.e., stored over $CaCl_2$.

(ii) at 10°C.
- $-CaCl_2$
- $+CaCl_2$

(iii) at O°C.
- $-CaCl_2$
- $+CaCl_2$

Receptive stigmas of previously emasculated and bagged flowers were pollinated with pollen stored under different conditions. The success of pollination as indicated by the number of fruits formed is shown in Table III.

TABLE III

PERCENTAGE FRUIT SET OBTAINED WITH POLLEN STORED FOR DIFFERENT PERIODS (25 flowers used for each test)

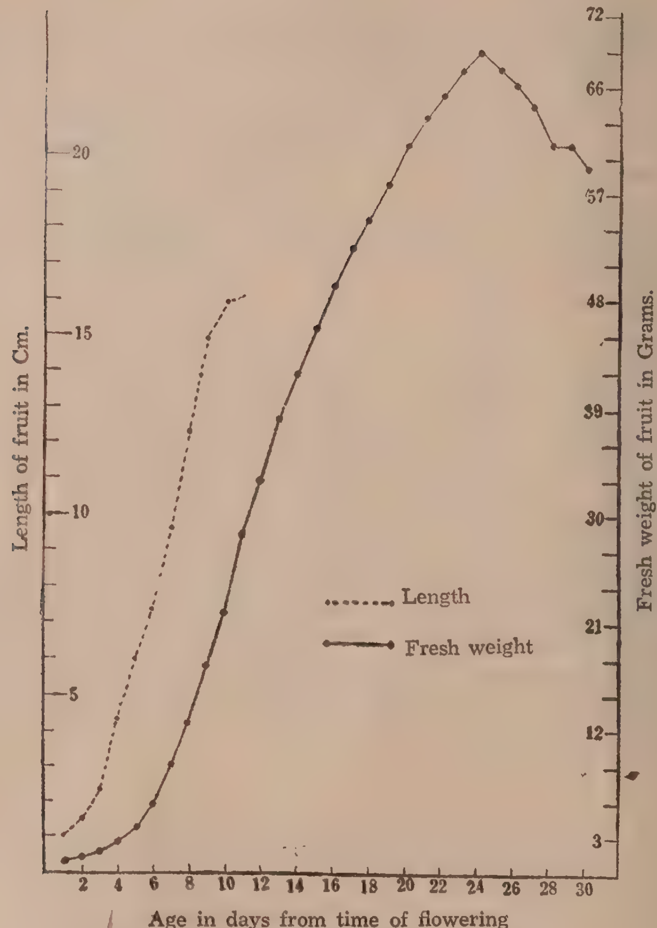
Storage condition		% set with pollen stored for					
		24	48	72	96	120	144 hours
Room Temp.	$-$ $CaCl_2$ ..	36	—	—	—	—	—
(30° C.)	$+$ $CaCl_2$ ..	48	—	—	—	—	—
10° C.	$-$ $CaCl_2$ ..	84	68	48	—	—	—
	$+$ $CaCl_2$ ..	84	72	56	44	20	—
Ice-chest	$-$ $CaCl_2$ ..	88	84	68	52	36	—
Temp. (0°C.)	$+$ $CaCl_2$ ..	84	84	72	68	56	20

The pollen lost its viability and vitality to set fruit considerably within 24 hours when stored at room temperature. That stored at 10°C. showed a fairly good retention of viability even after 96 hours. The best results were obtained, however, with pollen stored over calcium chloride and kept at O°C. (ice-chest temperature).

10. *Fruit development*:

The daily growth of the fruit was studied in the variety "P. 13". Measurements were made of the length of 10 growing fruits at 24-hour intervals from the time the withered corolla was shed until growth ceased. The distance between the apex of the fruit and its base, i.e., the point of union with the stalk, was taken as the length. Growth was rapid and by about the tenth day the fruit reached its maximum length (Text-fig. 13).

To have an idea of the fresh weight of the fruit at different stages of growth, 30 flowers were selfed and labelled and one fruit was harvested daily, the first being picked 24 hours after pollination. To ensure uniformity the fruit was cut at the point of fusion with the stalk and the fresh weight was determined. The maximum weight was reached on the 24th day. Thus, the fruit ceased to grow in length long before the maximum weight was attained (Text-fig. 13).



TEXT-FIG. 13

GRAPH SHOWING THE DAILY INCREASE IN LENGTH AND WEIGHT OF FRUIT

11. *Effect of placement of pollen on fruit shape*:

During the course of the study it was observed that in some varieties normally producing straight fruit, the fruit was sometimes unusually curved. Some such curved fruits showed insect damage, while others did not. In the latter, seed was not set uniformly in all the locules of the fruit suggesting that it might be due to uneven distribution of pollen on the stigmatic lobes. To verify this assumption the following test was carried out: the variety "P. 13" with straight fruit was chosen; some flowers were

artificially pollinated in such a way that all the five stigmatic lobes received the pollen grains. In some others, only one or two of the lobes were pollinated the remaining ones being removed from the stylar column. Normal straight fruits were produced by those flowers all the stigmatic lobes of which received the pollen, and curved ones resulted from the other treated flowers (Plate I, Fig. 5; Text-figs. 10-12). Dissecting the curved ones, seeds were seen to have developed in one to two of the locules only (Text-fig. 11). The curvature of the fruit appeared to be due to unequal development of the sides of the fruit indicating an effect of the placement of pollen on the shape of the fruit.

Discussion

The various observations made during the course of the study and their practical importance are discussed under the following heads:

The Flower:—The development of the flower, anther dehiscence and pollen dispersion were in all essentials similar to those reported by Vijayaraghavan and Wariar (1946) and Purewal and Randhawa (1947). The flower bud development was also studied by McGinty and Barnes (1932) in America, but unfortunately their paper in original was not available for reference. Purewal and Randhawa (1947) observed that the size of the pollen ranged from $672{\cdot}82\mu$ to $848{\cdot}48\mu$, varying with variety. In the present study, however, very little varietal difference was noticed, the diameter of the pollen grain averaging 160μ excluding the spines, and about 192μ inclusive of them. The stigma was found to be receptive 12 hours prior to anthesis and under the conditions of the experiment it retained its receptivity to an extent even on the day following flowering. Purewal and Randhawa (1947) reported that it was not receptive at least 20 hours before the opening of the flower.

The size of the flower bud a day prior to anthesis was seen to be a useful criterion in determining the right stage of the bud for emasculation purposes. This size also appeared to be a varietal character (Table II; Plate I, Fig. 2). A similar feature was recorded in the "brinjal", *Solanum melongena* L., by Pal and Singh (1943) and other workers.

Cross-pollination was the general rule under field conditions, failing which self-pollination occurred by the mechanical twisting of the petals and deposition of pollen on the stigmatic surface. This confirms the author's previous findings (Venkataramani, 1945). Purewal and Randhawa (1947) also noted that both self- and

cross-pollination can take place. There was no indication of selective fertilisation such as that observed by Breznev (1939) in the tomato. He concluded that the egg-cell exercised a selection of pollen biologically most compatible with it. In the present study, however, the early germinated pollen, whether self or foreign, appeared to reach the ovule first and effect fertilisation.

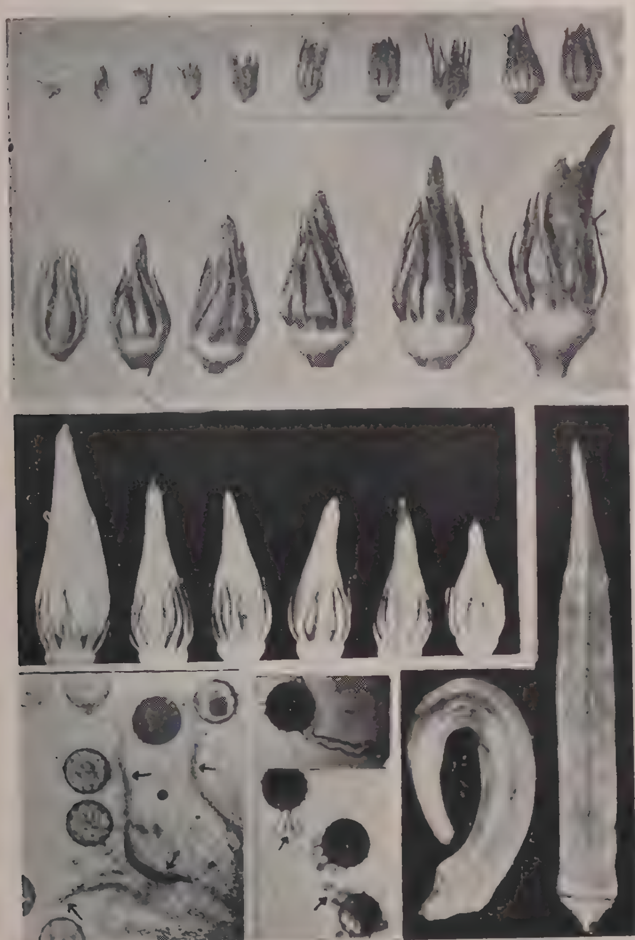
Natural cross-fertilisation: — In a crop in which cross-pollination can freely take place under field conditions, an understanding of the extent of natural cross-fertilisation that occurs is of fundamental importance in the improvement of varieties, as the amount of such crossing will influence the methods employed in breeding and maintaining pure lines. An attempt was, therefore, made to determine the extent of crossing taking place in the small experimental plots. A high percentage of crossing (31·7%) was noticed when two varieties were grown in close proximity to each other and about 8 per cent. occurred at a distance of 34 feet showing that seed for experimental purposes should not be collected without controlled pollination. Purewal and Randhawa (1947) also reported a high percentage of crossing ranging from 4 to 18·75. According to Beattie (1940), each of the varieties of this vegetable should be separated from the other by at least one quarter mile in order to prevent mixing. The same distance has been suggested as the minimum for growing different varieties and strains of the onion for the production of certified seed (Sparks and Binkley, 1943). These and the findings of other workers point out the importance of spatial isolation of seed crops in the case of the normally cross-pollinated vegetables. The four main variables affecting cross-pollination, viz., (i) the breeding system of the species, (ii) isolation distance, (iii) varietal mass and (iv) the pollinating agent, have been discussed by Bateman (1947). As expressed by him, there is very little work available entailing a systematic study of these four factors, and there is a need for such a study of the independent effects of these factors and their interaction.

Longevity of the pollen: —The "bhendi" pollen grains ordinarily remained viable for only 24 hours. They, however, retained their viability for greater periods when stored over calcium chloride and kept at low temperatures. The short life of pollen and the beneficial effects of refrigeration in the retention of viability were also observed in the case of the cotton, a plant closely related to the "bhendi" (Harrison and Fulton, 1934). There is evidence in many other crops too that the longevity of the pollen can be

PLATE I

1

2



3 4 5

FIG. 1: The different stages of flower development in *Hibiscus esculentus;* FIG. 2: Mature flower buds of six varieties, (*left to right*) Long Green W.V., Long Green R_8P_5, Podugu, P. 13, Mullu and Pal. Note the variation in size; FIG. 3 and 4: Germinating pollen grains. The arrows indicate pollen tubes in the spread stigmatic tissue. FIG. 5: The normal and an abnormally curved fruit of the variety "P. 13".

greatly prolonged under certain conditions of storage (Maheshwari, 1944).

It must be recognised, however, that mere germination is not the sole criterion of the usefulness of the stored pollen, for it is known that at least in some grasses the pollen loses its capacity to fertilise within a short time although it may be able to germinate even after a month (Darlington and La Cour, 1942). Therefore, pollination of the receptive stigma with the stored pollen and observation of fruit and seed production should be a better criterion for viability, though it would be more time consuming, than the empirical and artificial method of germinating on a slide. This method was adopted and it was found that pollen stored over calcium chloride and kept at the ice-chest temperature did not only retain its germination capacity, but was also capable of setting fruit for a period of six days (Table III).

Fruit development:—The "bhendi" fruit is characterised by a very rapid growth rate and in a variety under observation a length of 16 cm. was reached in ten days. The fruit increased in length at a slow rate for the first three days, thereafter the growth was very rapid (Text-fig. 13). There may be slight differences in the actual time taken by the fruit to attain its maximum size in different varieties, and climatic conditions may also influence the daily rate of growth. Culpepper and Moon (1941) found that in one and the same variety the fruit grew to a length of 3·92 cm. at 47·5°F., and 16·66 cm. at 72·5°F., in ten days, while at 82·5°F. a maximum length of 17·3 cm. was reached in only seven days.

The fresh weight of the fruit was not directly proportional to the length and the maximum length was reached long before the maximum weight was attained (Text-fig. 13). Culpepper and Moon (1941) also observed that the fruit reached a stage considered unpalatable long before the maximum amount of dry matter had accumulated in it. Although detailed investigations were not undertaken by the present author, it was the general observation that in all the six varieties the fruit was tender and fit for picking as a vegetable in six to eight days from the time of flowering.

Another interesting finding that needs mention is the curling of the fruit caused by the unequal deposition of pollen on the stigmatic lobes (Plate I, Fig. 5; Text-figs. 10-12). A similar effect of the placement of pollen on the shape of the watermelon fruit has been reported by Mann (1943).

Acknowledgement

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On Disjunction Lattices[1]

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ABSTRACT

This is a study of disjunction lattices, that is distributive lattices satisfying the Wallman disjunction property. Characterizations of disjunction lattices in terms of their ideal structure and also of Boolean algebras as special disjunction lattices are obtained.

In this note two new characterizations for disjunction lattices respectively in terms of the properties of the principal μ-ideals and the minimal-prime μ-ideals in L are obtained. Using these, some characterizations for Boolean algebras are deduced: the connection of these results to an earlier result of the author, as well as that to a theorem of Michiura is pointed out.

Throughout it will be supposed that L is a lattice with 0 and 1. Notations and terminology employed will be generally as in [7; Chapter III] (first number in square brackets refers to Bibliography given at the end).

Definition 1. A lattice L is said to be a *disjunction lattice* if it is distributive and has the property

(P): given any two distinct elements a, b, of L there exists an element c such that one of ac, bc, is 0, while the other is not [8; p. 115]. The dual of a disjunction lattice is a dual-disjunction lattice.

Remark 1. The disjunction property (P) is equivalent to the following weaker form of the same property (which is more convenient in practice).

(P′): given any two elements a, b, of L with $a <, \neq b$ there exists an element c such that $ac = 0$, $bc \neq 0$. For, let (P') hold,

1. The principal results of this note (Theorems 2 and 3) were essentially given before in the thesis of the author forming the basis for award of the Ph. D degree (1951) of this University.

and $a, b,$ be two distinct elements of L; then $a+b \neq$ at least one of $a, b,$ — say a. Since $a <, \neq a+b$, there is by (P'), a c with $ac = 0$ $(a+b)c \neq 0$; but $bc = ac + bc$ (since $ac = 0$) $= (a+b)c \neq 0$; hence (P) holds. The reverse implication is obvious.

In particular it results, that in a disjunction lattice if $a \neq 1$, there is corresponding to the pair $(a, 1)$, an element c with $ac = 0$, $c = 1 \cdot c \neq 0$, so that

The ideal II_a of a disjunction lattice is the zero α-ideal 0_a (i.e., the set consisting of the element 1 alone).[2]

Remark 2. If L be a Boolean algebra, $a, b,$ elements of L with $a <, \neq b$, and a' the complement of a, then $a' b \neq 0$ (else $b < (a')' = a$, which is impossible). Hence

A Boolean algebra is a disjunction lattice (and dually a dual-disjunction lattice).

Next one has:

Theorem 1. A distributive lattice L having an additive basis (m) of minimals[3] (or atoms) m is a disjunction lattice.

Proof. Let $a, b,$ be any two elements of L with $a <, \neq b$. It is clear from the hypothesis that there exists an atom m such that $m < b$, $m \nless a$. It follows that $am = 0$ (since m is an atom) and $bm = m \neq 0$. Hence L is a disjunction lattice.

Remark 3. The above theorem shows immediately that the lattice $L(R)$ of close sets of a T_1-space R, is a disjunction lattice. For it is plain that $L(R)$ has an atomic basis composed of one-pointic sets. Thus the class of disjunction lattices is much wider than the class of Boolean algebras.

Lemma 1. A distributive lattice L is a disjunction lattice, if and only if, distinct principal μ-ideals $P_\mu(a)$ of L have distinct product-complements $P'_\mu(a)$.

Proof. If L is a disjunction lattice, $a, b,$ in L, $a \neq b$, there exists a c with $ac = 0$, $bc \neq 0$. Hence c in $P'_\mu(a)$ but c not in $P'_\mu(b)$, whence $P'_\mu(a) \neq P'_\mu(b)$. On the other hand if L is not

2. II_a is the set of all elements of L with product-complement 0: II_a is an a-ideal [6; p. 380].

3. An element m of L is called a minimal (or an atom) if $m \neq 0$ and $a < m$ implies $a = 0$ or m,

a disjunction lattice, there exists in L a pair of distinct elements a, b, with the property: $ax = 0$ implies and is implied by $bx = 0$; so that $P'_\mu(a) = P'_\mu(b)$ $(a \neq b)$.

Theorem 2. A necessary and sufficient condition for a distributive lattice L to be a disjunction lattice is that every principal μ-ideal $P_\mu(a)$ of L be normal (i.e., $P''_\mu(a) = P_\mu(a)$).

Proof. The condition is necessary. Let L be a disjunction lattice. If possible let $P_\mu(a)$ be not normal so that[4] $P_\mu(a) \subset, \neq P''_\mu(a)$; then an element b can be chosen from $P''_\mu(a)$ with b not in $P_\mu(a)$ (so that $a + b \neq a$). Since $P_\mu(a) \quad P_\mu(a + b) \subset P''_\mu(a)$, on taking product-complements it results:[5] $P'_\mu(a) \supset P'_\mu(a + b) \supset P'''_\mu(a)$; but $P'''_\mu(a) = P'_\mu(a)$.[6] Hence $P'_\mu(a) = P'_\mu(a + b)$ with $a \neq a + b$, which contradicts Lemma 1. Thus $P_\mu(a)$ is normal.

The condition is sufficient. For, assume it holds in L. The relation $P'_\mu(a) = P'_\mu(b)$ implies, since $P_\mu(a)$, $P_\mu(b)$, are normal by assumption, the relation

$P_\mu(a) = P''_\mu(a) = P''_\mu(b) = P_\mu(b)$, i.e., distinct principal μ-ideals have distinct product-complements. Consequently by Lemma 1, L is a disjunction lattice.

Corollary 1. A disjunction lattice L is a Boolean algebra, if and only if, L is closed for product-complements.

Proof. For if L be a disjunction lattice closed for product-complements and a an element in L, then

$P_\mu(a'') = P''_\mu(a) = P_\mu(a)$ (by Theorem 2). Hence $a'' = a$, or every element of L is normal. Consequently L is a Boolean algebra. This proves one part; the other part is evident.

Note that since $II_a = 0_a$ in a disjunction lattice (Remark 1) the above result could also be inferred from [1; Theorem 10] (it might be remarked here that in [1] the symbol 0_a has been denoted throughout by merely the symbol 0).

Theorem 2 furnishes a characterization of disjunction lattices in terms of the product-complement theory of ideals. Now an alternative characterization involving the concept of the minimal-prime ideal will be obtained.

4. In general if A_μ is a μ-ideal $A_\mu \subset A''_\mu$ (cf. [7, § 12. 21]).
5. If $A_\mu \supset B_\mu$ then $A'_\mu \supset B'_\mu$ (cf. loc. cit).
6. $A'''_\mu = A'_\mu$ for any μ-ideal A_μ (cf. loc. cit.).

Definition 2. A μ-ideal $A_\mu \neq 1_\mu$ of L is called

(i) *maximal* if it is *properly* contained in no μ-ideal except 1_μ,

(ii) *prime* whenever it contains with a product ab always either a or b,

(iii) *minimal-prime* if it is prime and does not contain *properly* any prime μ-ideal.

The corresponding concepts for an α-ideal are defined dually.

In a distributive lattice L there is a simple relation between the maximal α- (or μ-) ideals and the minimal-prime μ- (or α-) ideals which is afforded by

Lemma 2. Complements[7] $c(M_\alpha)$ *of maximal α-ideals M_α of a distributive lattice L are minimal-prime μ-ideals m_μ and vice-versa; (and also dually).*

Proof. Since L is distributive, M_α is a prime α-ideal and hence $m_\mu = c(M_\alpha)$ is a prime μ-ideal; next if P_μ be a prime μ-ideal contained in m_μ, $c(P_\mu)$ is a prime α-ideal containing M_α. The maximality of M_α shows at once $c(P_\mu) = M_\alpha$ so that $P_\mu = m_\mu$, whence m_μ is minimal-prime. The other part is proved by reversing the argument.

Lemma 3. A distributive lattice L is a disjunction lattice, if and only if,

(i) *given any two elements a, b in L with $a <, \neq b$ there exists a maximal α-ideal M_α containing b but not a, or equivalently*

(ii) *every principal α-ideal $P_\alpha(a) \neq 1_\alpha$ is the product of all maximal α-ideals M_α containing it.* (cf. Wallman [8; Lemma 3]).

Proof. First, the equivalence of conditions (i) and (ii) will be established. Let (i) hold and N_α be the product of all maximal α-ideals M_α containing a, so that $N_\alpha \supset P_\alpha(a)$; if $N_\alpha \neq P_\alpha(a)$ there exists an element b in N_α with b not in $P_\alpha(a)$ (so that $ab <, \neq a$). By (i) there exists a maximal α-ideal M_α containing a but not ab; M_α cannot contain b since a in M_α, ab not in M_α, and M_α is an α-ideal. But by definition of N_α, since M_α contains a it results: $M_\alpha \supset N_\alpha \supset (b)$ which is a contradiction; hence $N_\alpha = P_\alpha(a)$, or (ii) holds. Con-

7. By complement (as distinguished from product-complement) of an ideal is meant the set of elements of L not belonging to it.

versely, if (ii) holds and $b >, \neq a$ then a is not in $P_a(b)$, and so there exists an M_a containing b but not a, i.e., (i) holds. Thus the conditions are equivalent.

Now let (i) hold. If $a <, \neq b$ there exists by (i) a maximal α-ideal M_a containing b but not a. Since M_a is maximal, $M_a + P_a(a) = 1_a$, and hence $ac = 0$, for some element c in M_a; but as b, c (and so also bc) are in M_a and $M_a \neq (0)$, $bc \neq 0$. Thus L is a disjunction lattice (Remark 1). Conversely let L be a disjunction lattice, and a, b, elements in L with $a <, \neq b$. Then there exists an element c with $ac = 0$, $bc \neq 0$. By Zorn's lemma there is a maximal α-ideal M_a containing bc, and hence also b; M_a cannot contain a since $abc = b.ac = 0$ and 0 not in M_a. Hence (i) holds and the proof is complete.

Lemma 4. A lattice L is distributive, if and only if, every principal α-ideal $P_a(a)$ $(\neq 1_a)$ is a product of prime α-ideals (or equivalently, the product of all prime α-ideals containing $P_a(a)$); (and dually, if and only if, every principal μ-ideal $(\neq 1_\mu)$ is a product of prime μ-ideals).

Proof. The "only if" part follows immediately from the classical result of Stone [5]: viz., every α-ideal $(\neq 1_a)$ of a distributive lattice is the product of all prime α-ideals containing it (and dually). On the other hand, the "if" part can be either inferred from the general results on representation of lattices due to Birkhoff and Frink [3; p. 309] or proved independently as follows.

Assume if possible $(a + b)c >, \neq ac + bc$. By hypothesis there is clearly a prime factor α-ideal P_a with $(a + b)c$ in P_a, $(ac + bc)$ not in P_a. Then $a + b$, c are in P_a; also since P_a is prime, one of a, b — say a, is in P_a. Hence ac is in P_a and therefore $ac + bc$ is in P_a (since P_a is an α-ideal) contradicting the choice of P_a. Hence $(a + b)c = ac + bc$, whence L is distributive.

Theorem 3. In order that a lattice L be a disjunction lattice, it is necessary and sufficient that every principal μ-ideal $\neq 1_\mu$ of L be the product of all minimal prime μ-ideals containing it.

Proof. First of all note that in view of Lemma 3, the given condition implies also the condition: L is distributive. The theorem will be therefore proved (see Lemma 3) if it can be shown that in a distributive lattice L the two statements

(i) every principal α-ideal $(\neq 1_a)$ is the product of all maximal α-ideals containing it, and

(ii) every principal μ-ideal ($\neq 1_\mu$) is the product of all minimal prime μ-ideals containing it, are equivalent. By virtue of Lemma 3, statement (i) can be reformulated as

(i_1) given two elements a, b, with $a <, \neq b$, there exists a maximal α-ideal containing b but not a. Again (by using a similar argument as in Lemma 3) it results that statement (ii) is equivalent to

(ii_1) given two elements a, b, with $a <, \neq b$, there exists a minimal prime μ-ideal containing a but not b.

The last two assertions are easily seen to be equivalent. For in fact, if M_a is a maximal α-ideal containing b but not a, $m_\mu = \mathrm{c}(M_a)$ is a minimal prime μ-ideal (Lemma 2) containing a but not b. Hence the proof is complete.

Theorem 4. In order that a lattice L closed for product-complements be a Boolean algebra, either of the following two conditions is necessary and sufficient:

(i) *every principal μ-ideal $\neq 1_\mu$ be the product of all minimal-prime μ-ideals containing it;*

(ii) *L is distributive and every principal α-ideal $\neq 1_a$ is the product of all maximal α-ideals containing it.*

Proof. Recall first that conditions (i) and (ii) are equivalent as demonstrated in Theorem 3. Next, if (i) holds, L is a disjunction lattice (Theorem 3), and hence a Boolean algebra (Corollary 1) since L is closed for product-complements. This proves the sufficiency of condition (i). Again if L is a Boolean algebra, L is a disjunction lattice closed for product-complements (corollary 1); hence by Theorem 3, (i) holds, whence the necessity of condition (i).

Remark 4. The above result in particular implies the following theorem of Michiura [4; Theorem 3]: *A complete lattice with completely distributive sums and products is a complete Boolean algebra, if and only if, every principal α-ideal is the product of all maximal α-ideals containing it.* It suffices to remark that since L has completely distributive sums, L is closed for product-complements; (note that the condition "L has completely distributive products" is redundant in the statement of the above theorem). It might also be mentioned here that the proof given in [4] is, unfortunately, invalid since it is based on the false relation (see p. 132) $M^{10}{}_a = M_a$ for a maximal α-ideal M_a ($M^{10}{}_a$ here denoting the *l.r.c.*

(last-residue class) of the *l.r.c.* of M_α). In any chain L (having more than two elements) for instance, the set M_α of all elements except 0 is a maximal α-ideal such that $M^{10}{}_\alpha = 0_\alpha = (1) \neq M_\alpha$.

Remark 5. In a disjunction lattice L, it was seen that the ideal $II_\alpha = 0_\alpha$ (Remark 1); (and dually in a dual-disjunction lattice $II_\mu = 0_\mu$). Also it has been shown elsewhere (see [2; Theorem 12]) that a complete lattice with $II_\alpha = 0_\alpha$, $II_\mu = 0_\mu$ is always a Boolean algebra. It therefore follows that a complete lattice L which is a disjunction as well as dual-disjunction lattice, is necessarily a (complete) Boolean algebra. On the other hand, there are incomplete, disjunction as well as dual-disjunction, lattices which are not Boolean algebras.

The following example (considered previously in [2]) will serve to illustrate this latter point. L is the lattice composed of all finite (including null) or countably infinite subsets of an uncountable set R together with the complements of the *finite* subsets. L is easily seen to be an incomplete, distributive lattice having an additive basis of minimals (viz., the one-pointic subsets of R) and a multiplicative basis of maximals (viz., the complements of the one-pointic subsets). By Theorem 1 (and its dual) L is a disjunction as well as dual-disjunction lattice. L is not, however, a Boolean algebra since complements of countably infinite subsets of R do not belong to L.

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Dielectric Constants by the Resonance Method

BY

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ABSTRACT

An A.C. mains operated apparatus for the accurate measurement of dielectric constants by the resonance method has been described. Results obtained with this apparatus are found to be highly reproducible and accurate as illustrated by a sample measurement in the case of carbon tetrachloride. Details of construction and operation have also been given so that the instrument may be easily duplicated.

The resonance method of determining dielectric constants using the basic principles of an oscillatory circuit is well known for its accuracy and simplicity. Recently this method has been developed to a great extent of accuracy by Le Fevre and Russell (1947) for the measurement of dielectric constants of gases and liquids. But the use of battery operated valves in their apparatus necessitating the use of accumulators for low tension and high tension supplies is not always convenient. Very accurate determinations of dielectric constants of liquids are conveniently made with the apparatus described here, which can be also easily duplicated.

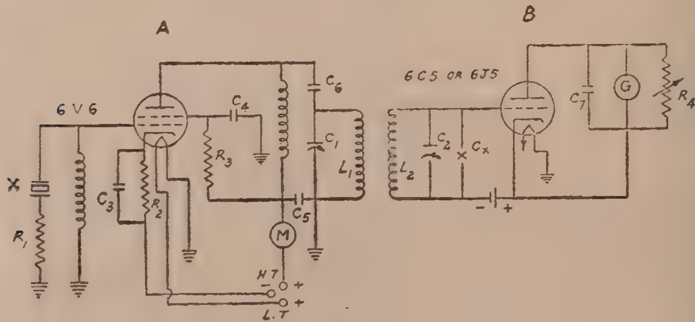

FIG. I

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Component values:

R_1 — 50,000 ohms. R_2 — 200 ohms, 2 watts. R_3 — 14,000 ohms, 2 watts.

R_4 — 10,000 ohms wire wound variable. C_1 — 500 mmfd. variable.

C_2 — 250 mmfd. variable, C_3, C_4 & C_5 — 0·01 mfd. Mica.

C_6 — 0·001 mfd. Mica. C_7 — 2 mfd. G — Galvanometer.

M — 0 to 100 Milliammeter.

Figure I shows the circuit employed for the purpose. One may consider here the factors that govern the accuracy of the measurements and limitations of the method. The circuit of Figure I may be schematically represented with inductance, capacities and resistances arranged accordingly. For accurate determinations of the resonance points of the circuit it is necessary to have the resonance curves obtained by plotting condenser settings against current intensity in the resonating circuit, as highly peaked as possible. To get at a symmetrical peaked resonance characteristic from circuit B of Figure I, it is essential to employ a loose inductive coupling and the circuit should have a high $Q = (\omega L/R)$. The resonant circuit of Figure I, B may be represented as shown in Figure II, where C_2 is the measuring condenser, C_x the test cell, r the resistance of the liquid used in the cell, l_2 the inductance of the leads to C_2, l_x the inductance of the leads to C_x. Writing the total capacity as C

$$C = C_2 (1 + \omega^2 l_2 C_2) + C_x (1 + \omega^2 l_x C_x + \frac{1}{\omega^2 r^2 C_x^2}) \quad \cdots \quad (1)$$

The equivalent resistance R of the circuit is given by

$$R = \frac{1}{\omega^2 C^2 r} \quad \cdots \quad (2)$$

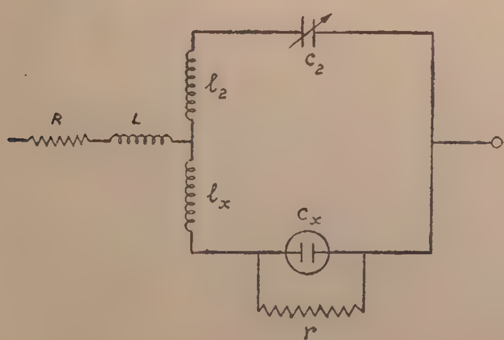

FIG. II

It is clear that the effects due to damping introduced by the resistance r of the liquid in the test cell may be minimised by using a higher value for C_2 and smaller value for C_x. The effect of inductance of the leads l_2 and l_x is to give 'false' capacity reading as has been shown by Lattey and Gatty (1929). Suitable corrections may be easily made as shown here and by choice of suitable layout of the apparatus it is possible to overcome the limitations indicated.

Apparatus: The circuit on the left hand side marked A in Figure I is the diagram of the crystal controlled oscillator using a mounted quartz crystal (× in Figure I) of frequency 1,000 Kcs/Sec., the oscillator valve being a 6V6 RCA operated with nearly 250 volts on plate. As the usual methods of rectifying alternating current supply often give only fluctuating direct current voltages, it was found essential to include a voltage stabiliser circuit to provide a stable voltage for the oscillator plate. The electronic voltage stabiliser circuit employed is quite a conventional one and has been found to be extremely efficient for the purpose (American Radio Relay League 1948) (Jen-Yuan Chien, 1947). Both the oscillator and resonator coils (L_1 and L_2) are wound on bakelite formers of about 3 inches diameter having about 50 turns of No. 20 D.S.C. wire so as to have a length to diameter ratio of 1:1.37 for this ratio minimises the temperature dependence of the inductance of the coil (Griffiths 1929). Further both the coils are doped with freshly prepared polystyrene so as to have little displacement of the wire of the coil on standing. The ends of the coils are brought to the respective terminals attached to their mounting ebonite pieces so that the length of the coil leads is kept as short as possible.

The resonator circuit consists of L_2 in parallel with the variable condenser C_2 and the test cell C_x, the resonance-indicating device being the valve voltmeter-part of this circuit using a type 6J5 valve and a ballistic galvanometer of very short period. A single 2-volt accumulator provides the grid bias voltage. A variable resistance is used in the plate circuit of the valve voltmeter to reduce the current through the galvanometer to a suitable value for purposes of observation. Radio frequency chokes are placed in the filament lines of the valves so as to prevent stray r.f. currents. The coils L_1 and L_2 are mounted coaxially and the whole assembly is kept inside a well-earthed thick aluminium case for r.f. screening. By means of suitably made perforations it is possible to have the control knobs of C_2 and R_4 on one side of the aluminium

panel. Slots are also made for reading the scales easily. A good shielded r.f. cable is used for connecting C_2 to C_x. The leads from the test cell C_x dip into two mercury cups made in a well fixed ebonite block. The test cell employed is of the Sayce-Briscoe type (Sayce and Briscoe 1925) silvered according to the recipe of Sugden (1933) and having a capacity of nearly 50 mmfd. The outer silver coating of the cell connects with the earth shield of the cable through one of the mercury cups while the inner coating connects with the insulated lead of the r.f. cable through the other cup. The test cell is immersed in a well-earthed water thermostat and it is supported rigidly by bakelite holders. All metallic objects in the neighbourhood of the cell are rigidly fixed and well-earthed. The variable condenser C_2 used for measuring the capacity change of the test cell is a rigidly mounted condenser with ball bearings on either side and is fitted with two sets of tuning-drive arrangement coupled together and consisting of a set of toothed wheels and vernier dial.

Calibration: The oscillator is first adjusted by varying the capacity of the condenser C_1 until a position is found in which the plate current as indicated by the milliammeter M is at a minimum. The test cell C_x is removed and the shape of the resonance curve is found by plotting the galvanometer deflection against the settings of the condenser C_2 in arbitrary units. The distance of coupling between the two coils L_1 and L_2 is adjusted by placing the oscillator chasis at different distances until the shape of the resonance curve is a symmetrical, sharp-peaked one, approaching the usual theoretical curve. With the present arrangement this distance was found to be within 3 feet between the two coils. The oscillator is then fixed in this particular position. The variable condenser C_2 is next calibrated in arbitrary units to note that portion of the condenser settings which may be nearly linear and with which measurements may be conveniently made. This may be done by the method of Fairbrother (1933) using a small fixed ceramic condenser of about 5 mmfd capacity at the mercury cups and a precision condenser (General Radio Company Type 222). The lead correction for the test cell may be obtained by the method of Watson (1924). The exact resonance points are determined by tracing the crest of the resonance curve by rotating the condenser C_2 in steps of single vernier division in one direction and noting the corresponding galvanometer readings and finally by reading out from the curve, care being taken to rotate the condenser always in one direction only so as to avoid backlash.

This method of determining the exact point of resonance gives very accurate readings.

We find that the incorporation of an air-thermostat for maintaining a constant temperature of the whole assembly results in highly reliable readings. A small 'end-effect' of the cell is noticed, the magnitude of which does not affect the accuracy of the measurements (Le Fevre and Russell 1947). The 'zero capacity' is determined from the capacity readings of the measuring condenser C_2 when the test cell is filled with dry air and subsequently with pure benzene, using the formula:

$$C_o = \frac{(C_A\varepsilon - C_B)}{(\varepsilon - 1)} \qquad .. \quad (3)$$

where C_o is the zero capacity in arbitrary units of condenser C_2, C_A the reading of C_2 with the test cell with dry air, C_B with pure benzene and ε the dielectric constant of pure benzene at the working temperature, taken from the expression:

$$\varepsilon_t = \varepsilon_{15} + \frac{\partial\varepsilon}{\partial t}(t - 15) \text{ where } \varepsilon_{15} = 2\cdot292_{5\pm5} \qquad .. \quad (4)$$

$$\text{and } \frac{\partial\varepsilon}{\partial t} = -0\cdot0019_{8\pm3}$$

given by Hartshorn and Oliver (1929). The dielectric constant of a liquid (ε_S) is then given by

$$\varepsilon_S = \frac{C_o - C_S}{C_o - C_A} \qquad .. \quad (5)$$

where C_S is the capacity reading of the measuring condenser C_2 with the liquid in the test cell.

Materials used: Analar benzene was repeatedly shaken with concentrated sulphuric acid, washed with water, treated with a dilute solution of caustic soda, washed again, dried over calcium chloride and then with sodium wire. After fractionation the middle portion was frozen out and finally stored over sodium wire which retained its metallic lustre. Carbon tetrachloride (B.D.H.) was first washed with concentrated sulphuric acid then with water, caustic soda solution and again with water. It was fractionated after drying over anhydrous potassium carbonate. It was finally dried with potassium hydroxide sticks.

Measurements: The results of measurements made with the apparatus described here are shown below. In this instance, a determination of the dielectric constant of pure carbon tetrachloride was undertaken so as to compare our value with that recorded in the literature. The present measurements were made at a temperature of 40°C ± 0·005°C. The final readings in arbitrary units given here were taken after applying all proper corrections for condenser non-linearity and leads.

Capacity with dry air = 11,370·1 divs. Capacity with benzene = 7,330·3 divs. Zero capacity (calculated) = 14,620 divs. Capacity with carbon tetrachloride = 7,477·1 divs. $\varepsilon_{40} = 2 \cdot 1978$.

With the large measured capacity displacements the significance of the fourth decimal place of the dielectric constant reported here may be realised. The value reported here is in excellent agreement with the values reported in the literature, thus proving the reproducibility and accuracy of the results obtained with this apparatus.

TABLE I

THE DIELECTRIC CONSTANT OF CARBON TETRACHLORIDE
TEMPERATURE 40°C. ± 0·005°C.

Author		Values	References
Goss, F. R.	..	2·1999*	*J.C.S.* 1341 (1933)
Miller, J. G.	..	2·1978 ± 0009	*J.Am.C.S. 64,* 117—121 (1942)
Cowley, C. G. and Partington, J. R.	..	2·197	*J.C.S.* 130 (1937)
Present work	..	2·1978	

* Value reduced to vacuum as unity

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Food of the Sardines of Madras Coast *

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ABSTRACT

Stomach contents of 189 specimens of *Sardinella brachysoma*, 248 *S. kanagurta* and 324 *S. melanura* were analysed of which the latter was divided into two size groups according to their size and the difference in their diet discussed.

While there are indications of particulate as well as filter feeding, the sardines are found to be filter feeders. That to some extent they are capable of selective feeding is shown by the total absence of teleosteans and the predominance of crustaceans in their diet. The absence of this species in certain months of the year cannot be explained until we know more about their migratory habits and also the seasonal distribution of the food organisms in the environment.

Though the clupeids enjoy a world wide distribution in the tropical and sub-tropical waters, it is well-known that true sardines belonging to the genus *Sardina* are confined to the colder waters. A wealth of information regarding the food and feeding habits of the various species of sardines of the temperate countries have been made available through the works of Dunn 1892, Roule 1914, Lebour 1919, 1920, 1921, 1927, Swithinbank and Bullen 1913, 1914, Kishinouye 1907, Lewis 1929, Parr 1930 and Radovich 1952, to mention only a few. However, in the tropics the place of the true sardines is taken up by species of *Sardinella* and among the important species which contribute to the bulk of sardine fisheries of the warmer waters may be mentioned *Sardinella cameronensis*, *S. aurita*, *S. longiceps* and *S. fimbriata*. In India *S. longiceps* and *S. fimbriata* rank as the most valuable species along the West coast while in the South-east coast *S. gibbosa* occur in shoals. *S. brachysoma*, *S. kanagurta* and *S. melanura* are the Indian sardines which are commonly landed in Madras. Our knowledge of the food and feeding habits or other aspects of biology of these fishes is comparatively meagre and the Indian oil sardine (*S. longiceps*) is the only species whose biology has been studied fairly completely. Hornell 1910 investigated the feeding, migratory and

* Part of the thesis approved for the degree of Master of Science of the University of Madras.

spawning habits of *S. longiceps, S. lile,* and *S. fimbriata* from Madras coast and later in 1924 Hornell and Nayudu gave a comprehensive account of the biology of *S. longiceps.* The food and feeding habits of *S. gibbosa* of the Madras Presidency and the Gulf of Mannar were made known by Devanesan 1932, and Chacko 1946-1949. Devanesan and Chidambaram 1948 have briefly dealt with the food of the sardines of the Madras Presidency.

It is hoped that the present paper on the food of *S. brachysoma, S. kanagurta* and *S. melanura,* the result of over 24 months' study, will add to our knowledge of Indian sardines. Though the fishes do not form the chief source of income for the fishing community, they are landed in considerable numbers in Madras. The specimens studied were all caught in the inshore waters of Chepauk, Triplicane and San Thome areas of Madras coast and the observations made are based on the analysis of stomach contents of adult specimens except in the case of *S. melanura* where the specimens obtained varied appreciably in size and were therefore divided into two size groups for the sake of convenience. Methods followed in the analysis of the stomach contents were those described by the author elsewhere (1951).

Family — *Clupeidae*
Sub-family — *Clupeinae*
Genus — *Sardinella*
Species — *Sardinella brachysoma* (Blkr)
Sardinella kanagurta (Blkr)
Sardinella melanura (C.V.)

Sardinella brachysoma. (Blkr).

Tamil name: 'Nonalai'

Met with in fairly good numbers in the catches during September and October and were abundant in the months of April and May. Stomach contents of 189 specimens whose standard length ranged between 4·8″ and 8·7″ were examined.

The analysis of the stomach contents is given in Table I, which shows that while the fish depends chiefly on crustacea for sustenance it is the small crustaceans such as the entomostrucans that constitute the major part of its crustacean diet. Copepods belonging to species *Euterpina, Oithona, Pseudodiaptomus* and *Acartia* were the dominant constituents of the entomostrucan diet. The larger items of food were made up of schizopods like *Mesopodopsis orientalis,* larval forms of squilla, lucifer spp and *Acetes erythraeus.* Other minor items were polychaetes, larval bivalves and algae. In

view of the fact that the fish was available only during four months in the year, no effort is made to study monthly fluctuations in the various food items.

Sardinella kanagurta (Blkr)
Tamil name: 'Seedai'

Were available during August, September, October and January reaching a maximum in September and October and 248 specimens were examined.

The examination of the stomach contents revealed that the diet of the fish was dominated by crustaceans of rather smaller size. Excepting in the case of a single specimen where the stomach contained a half digested teleostean fish, the diet of *S. kanagurta* was devoid of pescine item. Entomostruca such as the copepods belonging to species *Oithona, Euterpina* and *Macrosetella,* ostracods and larval cirripids were predominant in the stomach contents.

TABLE I
VOLUMETRIC PERCENTAGES OF THE FOOD COMPONENTS OF *SARDINELLA BRACHYSOMA* (Blkr)

	Months	Sept.	Apr.	Oct.	May
	Number of specimens examined ..	21	14	60	94
	Number of specimens with food contents ..	14	14	52	85
	CRUSTACEA ..	31·21	62·75	38·83	19·08
Larger crustacea	Acetes ..	12·14	1·25	3·82	1·72
	Mysidaceae ..	—	—	0·64	—
	Paguridae ..	—	—	—	0·40
	Anomuran larvae ..	—	—	0·41	—
	Squilla larvae ..	—	—	—	0·54
	Lucifer ..	—	—	0·09	1·25
	Other sergestids ..	1·64	—	—	—
Smaller crustacea	Copepoda ..	7·14	57·00	23·09	13·25
	Copepod nauplii ..	0·29	0·25	0·82	—
	Ostracoda ..	2·64	0·25	—	0·14
	Amphipoda ..	3·79	—	—	—
	Zoea larvae ..	0·43	4·00	1·14	0·57
	Crustacean remains ..	3·14	—	8·82	1·21
	Molluscan larvae ..	0·57	1·50	0·04	0·14
	Polychaeta ..	1·00	—	0·23	0·21
	Eggs ..	0·14	—	0·54	—
	Algae ..	1·14	—	0·50	0·43
	Sand particles ..	—	—	—	0·14

Decapods like penaeus larvae, young and larval palaemonids, larval anomurans and megalopa and zoea stages of brachyuran larvae, larval stages of squilla and sergestids like lucifer were the other crustacean items frequently met with. Bivalve larvae, polychaetes and algae were also met with in small quantities. Details of the analysis are presented in Table II.

Sardinella melanura (C.V.)
Tamil name: 'Kavalai'

This fish was available throughout the year except in the months of December when it was absent in the catches and in January when only stray ones were caught. The largest specimen that was procured measured 8·4″ while the smallest was 2·4″ in standard length. A total of 324 specimens were examined and these were divided into two size groups; those which measured 5″ and below being classified as 2 to 5″ size group and those measuring above 5″ being grouped as the 5 to 8″ size group. There were 222 individuals under the 5 to 8″ group and 102 under the 2 to 5″ group.

5 to 8″ size group:

Fishes of this group were obtained in plenty in the months of September and October and were available in small numbers in the other months of the year except in December when no fish of this species was available.

While appreciable quantities of large sized crustacea such as *Penaeus* sp., *Acetes erythraeus* and mysids like *Mesodopsis orientalis* were met with in the stomach contents frequently, the 5 to 8″ size group subsisted chiefly on the smaller crustacea. *Copepods, Pseudodiaptomus, Paracalanus, Eucalanus, Corycaeus, Oithona,* ostracods and larval forms of decapods appear to be the favourite food of the fish. The high percentage of copepods that formed the chief item of diet is noteworthy. Other items of food were the molluscan larvae Sagitta, Pycnogonida, and algae. Details of the analysis are presented in Table III.

2 to 5″ size group:

Occurrence of this group in the catches were restricted to the months of July, August and September and in the month of March a single specimen was obtained the analysis of the stomach contents of which has also been included in Table IV. The maximum number of this size group was caught in August.

TABLE II

VOLUMETRIC PERCENTAGES OF THE FOOD COMPONENTS OF *SARDINELLA KANAGURTA* (Blkr)

	Months	Aug.	Sept.	Oct.	Dec.	Jan.	Apr.
	No. of specimens examined ..	32	64	56	16	40	40
	No. of specimens with food contents ..	32	60	52	14	28	19
	CRUSTACEA ..	37·34	22·80	17·07	48·00	59·88	39·38
Larger crustacea	Acetes ..	—	—	—	—	1·43	0·68
	Penaeus larvae ..	—	—	3·41	—	0·07	0·21
	Palaemonidae ..	—	4·50	—	—	—	—
	Mysidaceae ..	—	—	—	—	0·64	—
	Paguridae ..	—	—	—	—	—	0·11
	Other anomura ..	—	1·00	—	—	—	—
	Lucifer ..	0·25	—	—	—	0·96	1·32
	Other sergestidae ..	—	—	—	—	—	0·11
	Squilla larvae ..	—	—	0·27	—	2·07	1·47
Smaller crustacea	Copepoda ..	23·75	7·80	1·09	48·00	53·00	31·63
	Copepod nauplii ..	1·00	0·10	2·18	—	—	—
	Ostracoda ..	—	0·65	1·36	—	0·11	0·53
	Amphipoda ..	—	0·55	—	—	0·07	1·11
	Megalopa ..	11·67	5·80	3·18	—	0·43	0·74
	Zoea ..	0·67	2·30	4·09	—	0·64	0·21
	Cypris larvae ..	—	0·10	—	—	—	—
	Crustacean remains ..	—	—	1·49	—	0·46	1·26
	Pycnogonida ..	—	—	0·18	—	—	0·78
	Polychaeta ..	0·17	—	0·09	—	0·29	0·89
	Bivalve larvae ..	0·33	—	0·36	—	0·93	0·68
	Eggs ..	1·00	0·2	0·09	—	0·43	0·63
	Algae ..	—	0·1	—	—	—	—
	Green matter ..	—	—	—	—	1·07	0·37
	Sand particles ..	—	—	0·40	—	1·04	0·11
	Teleostea ..	—	—	—	—	—	0·14

Table III

VOLUMETRIC PERCENTAGES OF THE FOOD COMPONENTS OF 5 TO 8″ SIZE GROUP OF *SARDINELLA MELANURA* (C.V.)

	Months	July	Aug.	Sept.	Oct.	Nov.	Jan.	Feb.	Mar.	Apr.	May	June
	No. of specimens examined	21	20	45	45	8	7	9	12	20	24	11
	No. of specimens with food contents	20	18	45	40	8	7	9	10	12	10	8
	CRUSTACEA	31·00	32·82	49·24	32·50	37·34	32·50	39·22	39·60	17·91	35·70	40·00
Larger crustacea	Penaeus	0·25	4·55	0·67	—	—	—	—	—	—	—	—
	Mysideceae	—	4·78	6·57	5·85	—	—	—	—	0·33	2·50	—
	Acetes	—	—	4·07	7·05	—	—	—	—	—	0·20	—
	Anomura	—	—	—	1·20	—	—	—	—	—	—	—
	Squilla larvae	—	—	0·07	3·65	—	—	—	—	—	1·60	—
Smaller crustacea	Copepoda	20·95	12·00	30·68	4·70	36·67	32·50	38·33	39·00	9·00	12·00	38·00
	Copepod nauplii	1·50	0·22	0·27	0·25	0·67	—	0·67	0·40	0·17	0·50	—
	Ostracoda	0·10	3·33	—	—	—	—	—	—	—	1·20	—
	Amphipoda	—	—	—	2·85	—	—	—	—	—	—	—
	Cypris larvae	0·10	—	—	—	—	—	—	—	—	—	—
	Megalopa	2·10	2·22	3·03	3·65	—	—	—	—	0·08	4·00	—
	Zoea	5·95	4·55	0·93	3·30	—	—	—	—	5·00	1·70	2·0
	Crustacean remains	—	1·17	3·0	—	—	—	0·22	0·20	3·33	42·00	—
	Bivalve larvae	—	0·62	0·31	0·10	—	—	—	—	0·58	0·7	—
	Pteropoda	0·15	—	—	0·10	—	—	—	—	—	—	—
	Sagitta	—	0·11	0·47	1·70	—	—	—	—	—	—	—
	Pycnogonida	—	0·11	0·40	—	—	—	—	—	—	—	0·50
	Eggs	—	0·17	0·10	0·20	—	1·00	0·67	1·6	0·5	—	—
	Algae	—	—	—	0·15	—	—	—	—	0·08	0·6	—
	Green matter	—	0·80	—	—	—	—	—	—	—	2·0	—

The 2 to 5″ size group appear to feed more frequently on vegetable matter than the 5 to 8″ group and small crustacea, mostly larval forms predominated in the stomach contents of the 2 to 5″ size group while appreciable quantities of larger crustacea were met with in the 5 to 8″ size group. The copepods consumed by the 2 to 5″ size group was great and there was an increase in the quantity of copepod nauplii taken when compared to the 5 to 8″ size group.

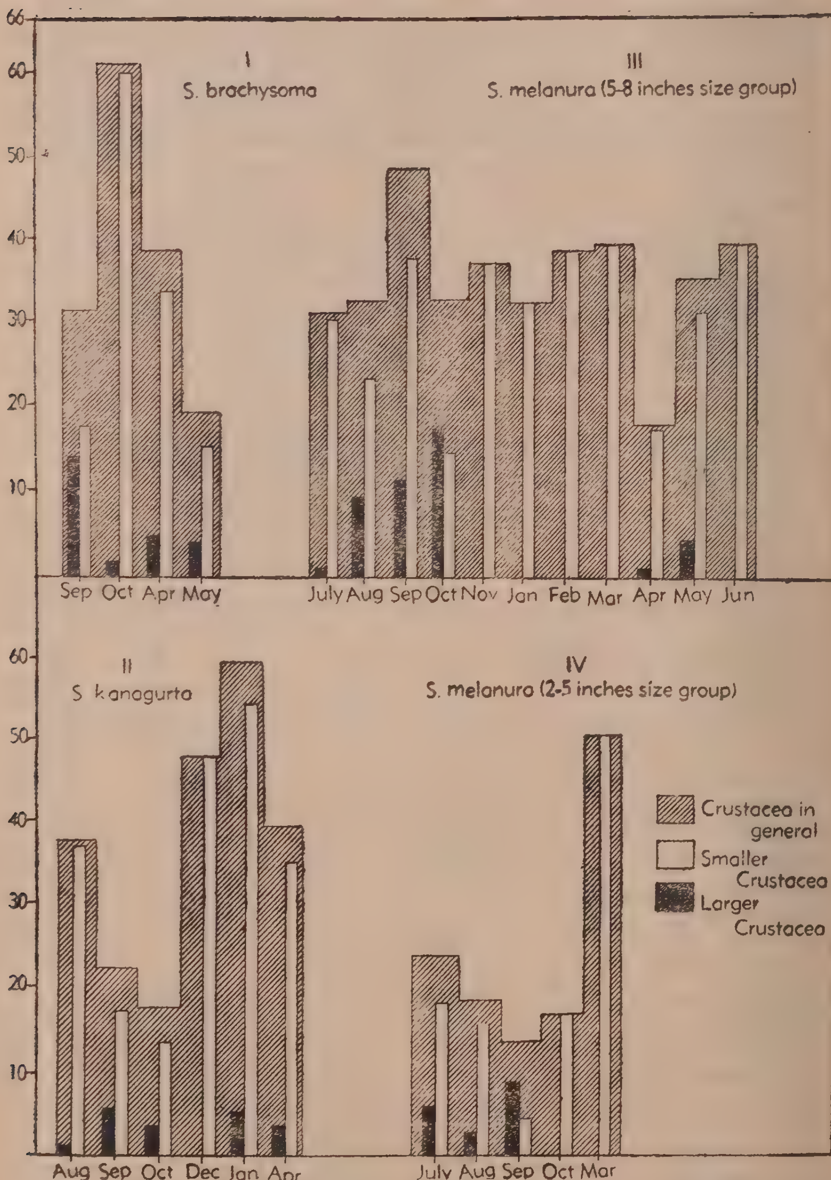
This difference in the quantity of the food taken by the two size groups may be due to the fact that the smaller size group are not capable of ingesting prey beyond a particular size.

Details of the analysis are presented in Table IV.

TABLE IV

VOLUMETRIC PERCENTAGES OF THE FOOD COMPONENTS OF 2 TO 5″ SIZE GROUP OF *SARDINELLA MELANURA* (C.V.)

	Months	July	Aug.	Sept.	Oct.	Mar.
	No. of specimens examined ..	24	45	27	15	1
	No. of specimens with food contents ..	20	38	27	13	1
	CRUSTACEA ..	23·90	18·46	13·57	16·99	60·00
Larger crustacea	Acetes ..	—	—	8·86	—	—
	Mysidaceae ..	—	2·78	—	—	—
	Lucifer ..	0·10	—	—	—	—
	Sergestid remains ..	5·60	—	—	—	—
Smaller crustacea	Copepoda ..	9·00	—	—	—	—
	Copepod nauplii ..	2·00	1·11	—	0·33	—
	Zoea ..	7·00	—	1·43	10·00	—
	Megalopa ..	0·20	0·07	—	—	—
	Bivalve larvae ..	—	0·64	—	—	—
	Pteropoda ..	—	0·07	—	—	—
	Sagitta ..	—	0·21	—	—	—
	Eggs ..	—	0·32	0·29	—	—
	Green matter ..	10·00	0·07	—	—	—
	Sand particles ..	0·10	—	—	—	—



Volumetric percentages of the smaller and larger Crustacea and Crustacea in general, in the three species of *Sardinella*

Discussion

The foregoing analysis of the food indicates that the three species of *Sardinella* can be described as surface feeders and may be called plankton predators.

While the size and quantity of the smaller organisms ingested indicate filter feeding, the occurrence of larger animals such as *Acetes* sp., *Mysis*, etc., which are of a more solid type than the plankton is suggestive of particulate feeding. The data described in the preceding pages and histograms (I, II, III, IV) show clearly that even though they are capable of feeding on organisms of larger consistency indicating particulate feeding, the major part of its food is constituted by the smaller planktons showing thus that essentially these fishes are filter feeders.

There has been considerable diversity of opinion as to whether the sardine is selective or non-selective in its diet. Many have reported on the predominance of different organisms in sardine collected at different places. Kishinouye (1907) showed that *Sardinops melanosticta* has a 'diverse appetite' and has expressed his belief that sardines can select to a certain degree by manipulation of the gill rakers. Velappan Nair (1951) describes the food of *Sardinella longiceps* as consisting of plankton with phytoplankton predominating. The stomach contents of the sardines described in the present paper is distinctly predominated by zooplankton. Hart and Wailes (1932) found that sardines collected off British Columbia contained greater volume of phytoplankton than anything else. But this was ascribed to the heavier concentrations of phytoplankton in the locality correlated with the filter feeding of the fish. The absence of teleostei in the stomach contents of the species under consideration is striking and that these fishes have been selective to the extent that they were partial to crustacea in general, cannot be denied. But any attempt to explain how far these fishes are capable of selective feeding and to what extent the occurence of crustacea or other organisms in the environment will influence the movements of these fishes would be merely speculative so long as we do not have a thorough knowledge of the distribution of these organisms as well as these fishes in our waters. As such it remains to be explained whether the absence of *Sardinella brachysoma* in the months of July, August, November, December, January, February, March, and June and *S. kanagurta* in July, November, February, March, May and June and *S. melanura* in the month of December has any correlation with their migratory habits or whether it has been due to the defects in the methods employed in capturing them.

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Inhibition Analysis

A Review with reference to the B Vitamins

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As science advances and as knowledge accumulates, man in his attempts at peering into the secrets of nature, brings into the field many new and ingenious methods of research. Three such new methods concerning intermediary metabolism are the tracer technique using labelled compounds, mutant methodology and inhibition analysis. Inhibition analysis has been defined as "the field of research employing competitive metabolite-analogue inhibitions in the study of biochemical reactions." (Shive 1950). Hence, for a clearer understanding of inhibition analysis, the terms "Metabolite", "analogue", and "competitive inhibition" should be explained.

Metabolite, Analogue and Competitive Inhibition :

Any chemical compound which is essential to the existence or the activity of living things, and thus plays a decisive role in metabolic reactions is usually called "essential metabolite," or more simply a 'metabolite'. It may be a vitamin or an hormone or an amino acid or any other compound which occurs naturally and is necessary in some phase of the life process. An "analogue" or "inhibitor" is any compound which is closely related in structure to the metabolite, but biologically antagonistic in action. If the metabolite is necessary for the multiplication of a bacteria, the analogue will arrest the growth producing bacteriostasis. If the metabolite is necessary for the normal growth of an animal, the administration of the analogue will cause all the symptoms of a deficiency of the metabolite. If the metabolite forms part of an enzyme system, the addition of the analogue will inhibit its activity. Any attempt at elucidating the pathways of metabolic reaction by making use of the structurally-similar inhibitor to block

the various functions of the metabolite constitutes inhibition analysis.

The antagonism between the metabolite and the analogue is "competitive" in nature. It has been found that, in a number of cases, a constant ratio exists between the amounts of the two substances which just counterbalance each other. This ratio is known as "the inhibition index." It is the ratio of the molecular concentration of the analogue to the molecular concentration of the metabolite necessary to obtain a defined inhibition. For example, the inhibition index between the analogue sulphanilamide and the metabolite para-amino benzoic acid for the complete inhibition of the growth of *E. Coli* is 3000 i.e. for any concentration of para-amino benzoic acid present in the medium, to produce complete inhibition of growth about 3000 times as much sulphanilamide is needed. If more of sulphanilamide is present, there will be complete inhibition. If the amount is less, there will be growth. Thus it will be seen that the deciding factor regarding inhibition or growth is not the absolute amounts of either of the two substances, but the relative ratio of their concentrations, i.e. the inhibition is "competitive". The inhibition index is just a measure of the potency of the inhibitor, the more potent inhibitors being characterized by a small inhibition index.

The analogues of B Vitamins:

Corresponding to the various members of the B Vitamins, a number of structurally similar inhibitors — the antivitamins — has been synthesized. They vary widely in their "antivitamin" potencies as given by their inhibition indices. For example, with 3, 4 — Ureylene cyclo-hexyl butyric acid and biotin, the inhibition index is as high as one million, while in the case of aminopterin, a powerful antagonist of folic acid characterised by an unusual potency, the inhibition index under certain conditions is no more than 0·5. Some of them like pyrithiamine or aminopterin are capable of calling forth with great speed in animals all the symptoms of extensive deficiencies of the corresponding vitamins, the deficiency syndromes being of more pronounced intensity and sharper definition than those produced with mere vitamin-deficient diets; while the other antivitamins produce only some of the symptoms of vitamin deficiencies. They also vary widely in their activity towards various species; some of them like pyrithiamine being active antivitamins for almost all the organisms tested, while others like pantoyl taurine or pyridine 3-sulphonic acid are

THE B VITAMINS AND THE CORRESPONDING ANTIVITAMINS

Vitamin	Inhibitor	Structural alteration	Biochemical System affected	Approximate Inhibition Index	Reversal	Reference
Thiamine	Pyrithiamine	$CH=CH$ for S	Mice, rats and bacteria	10 in animals 700 in bacteria	Competitive and complete	Woolley & White 1943
	Oxythiamine	OH for NH_2	Rats and chicks	—	Reversible	Soodak & Cerecedo 1944 Daniel & Norris 1949
Riboflavin	Isoriboflavin	Shift in position of CH_3	Rats, not bacteria	—	Do.	Emerson & Tishler 1944
Nicotinic acid	Pyridine 3-sulphonic acid.	SO_3H for COOH	Some bacteria	—	Do.	McIlwain 1940
	β-acetyl pyridine	$COCH_3$ for COOH	Mice	—	Do.	Woolley 1945
Pantothenic acid	Pantoyl taurine	SO_3H for COOH	Bacteria only	1000 to 24000	Competitive and complete	Snell 1941
	Phenyl pantothenone:	COC_6H_5 for COOH	Bacteria	of the order of 1000	Complete reversal	Woolley & Collyer 1945
	w-methyl pantothenic acid	CH_3 for H	Bacteria and rat	100	Complete	Drell & Dunn 1951
Pyridoxine	Desoxypyridoxine	H for OH	Chick, dogs and bacteria	2 to 4	Reversible	Ott 1946
	Methoxypyridoxine	OCH_3 for OH	Chicks and dogs	—	Do.	Karnofsky et al. 1950
Biotin	Desthiobiotin,	2H for S	Some bacteria	—	Do.	Lilly & Lionian 1944
	3, 4, Ureylene cyclohexylbutyricacid	2C for S	Bacteria	1 million	Do.	English et al. 1945
P-amino benzoic acid	Sulphanilamide and derivatives	SO_2NH_2 for COOH	Bacteria	3000	Competitively reversible	Woods 1940
Folic acid	Aminopterin	NH_2 for OH	Bacteria, animals, humans	2 to 5 in bacteria	Reversible in bacteria; Irreversible in animals	Franklin et al. 1948 Oleson et al. 1948
Inositol	Gammaxene	6Cl for 6H	Fungi, plants yeast	—	Reversible in some cases	Kirkwood & Philips 1946 Burton et al. 1946

active only in the case of bacteria and not for animals. Even among bacteria, they are active against only some and not all. Thus they are characterised by a high selectivity in their action. Wide variations are also exhibited in the abilities of the various vitamins to counteract the effects of corresponding antivitamins. The antagonistic effects of some of them like the sulphonamides, pantoyl taurine or pyrithiamine can be reversed in a competitive manner over a wide range of concentrations by the corresponding vitamins. But with aminopterin or gammaxene on the other hand, while there is reversal at low concentrations of the inhibitor, the toxic effects of the higher concentrations of the inhibitor cannot be reversed even by large doses of the vitamins. In the accompanying table are given the various particulars about some of the more important antivitamins.

Criteria for an "Antivitamin" effect :

The surest indication that the antagonistic effects produced by a structural analogue are due to competition of that analogue with its metabolite (the vitamin) is the ability of the vitamin to reverse completely the action of the agent. The evidence is strongest when there is a constant inhibition index over a wide range of concentrations. This is true in the case of the antivitamins, sulphonamides, pyrithiamine and pantoyl taurine. But cases are known where the effects of the antagonists are irreversible; but still the antagonists should be considered as antivitamins since they produce all the symptoms of deficiencies of the corresponding vitamins. Aminopterin constitutes a typical example of the latter class of the antivitamins. It produces quickly all the symptoms of extensive folic acid deficiency in animals, but the action cannot be reversed by folic acid. Hence, while the counteraction by the correpsonding vitamin is an excellent criterion, it is not always a sufficient one.

Hypothesis concerning mechanism :

Various hypotheses have been put forward to explain the mode of action of the inhibitor. But the one that is generally accepted and which also explains the existing facts best is given here. This hypothesis derives its inspiration from considerations of certain principles of enzymology. In enzymic reactions, it is well known that the substrate or metabolite combines initially with the enzyme, a specific protein to form an enzyme — substrate complex which then undergoes various metabolic reactions giving

rise to the products, the enzyme or protein being regenerated. According to the present hypothesis, the vitamin or any other metabolite functions by combining with a specific protein to form a metabolite-protein complex which then undergoes various metabolic reactions. So that the metabolite may combine with the specific protein, it should have certain structural features to satisfy the specificity of the protein. Because the inhibitor has a closely related structure, it is also able to combine with the protein to yield an inhibitor-protein complex. But this inhibitor-protein complex is unable to proceed through the subsequent metabolic reactions, because of the slight differences in the structures of the inhibitor and the metabolite ; the differences coming into play at this stage. Thus the inhibitor by combining with a portion of the protein to form the metabolically inert inhibitor-protein complex, deprives the metabolite of that portion of the protein for effective combination, and thus causes inhibition.

This hypothesis also explains competitive inhibition on the basis of the reversibility of the metabolite-protein and inhibitor-protein complexes, and on the relative effinites of the metabolite and the inhibitor for the protein. Usually the metabolite has a greater affinity for the protein than the inhibitor so that a lesser amount of it is required to saturate the protein. Therefore, the inhibition index is generally greater than one, though the reverse may be true with regard to aminopterin and folic acid where the inhibition index is less than one. As the concentration of the analogue is raised in the presence of a constant amount of the metabolite, a point is reached at which the protein, in accordance with the law of mass action, unites with the former, rather than with the latter substance, and inhibition of the metabolic function results. If the amount of metabolite is then raised the analogue is dislodged and normal function is resumed. If an analogue is so constituted that it would combine more or less irreversibly with the protein, an inhibitor would result which would call forth all the signs of deficiency in the organisms, but these manifestations cannot be reversed with increased doses of the metabolite. This may explain the behaviour of aminopterin at higher concentrations.

The only evidence for this hypothesis with reference to vitamins is with biotin (Dittmer and du Vigneud 1944). Biotin combines with the protein avidin to form biotin-avidin complex. Biotin-sulphone, an analogue of biotin is able to displace biotin from the biotin-avidin complex combining in its stead.

On the basis of the above hypothesis, by the application of the law of mass action, Shive and Macow (1946) have derived mathematically that, for a defined inhibition of growth in a constant period of time, the inhibition index should be a constant.

Mode of action of certain antivitamins :

The mode of action of many antivitamins have been studied. At least four of them have been shown to inhibit the enzymic conversion of the vitamins into their more active functional derivatives. For example, phenyl pantothenone inhibits the formation of coenzyme A from pantothenic acid (Novelli and Lipmann 1948), pyrithiamine inhibits the conversion of thiamine into cocarboxylase (Woolley 1951), sulphonamides suppress the biosynthesis of pteroylglutamic acid from p-amino-benzoic acid (Miller 1944), while aminopterin not only prevents the formation of citrovorum factor from folic acid, but also competes with citrovorum factor for various enzyme systems (Nichol and Welch 1950). The mechanism of action of desoxypyridoxine seems to be different in that it is first phosphorylated to desoxypyridoxine-phosphate which then competes with pyridoxal-phosphate for the apoenzyme in decorboxylases (Umbreit and Waddell 1943).

Reversal of inhibition by structurally unrelated substances :

The action of some antivitamins is overcome not only by the structurally analogous vitamin, but also by various structurally unrelated substances. This phenomenon is important because it throws considerable light on the metabolic functions of the vitamins. For example, the inhibitory effect of sulphanilamide on *Streptobacterium plantarum* is completely overcome not only by p-aminobenzoic acid, but also by peteroylglutamic acid (Lampen and Jones 1947). But in this case the antagonism is no longer competitive. The growth is dependent entirely on the amount of pteroylglutamic acid added and when a sufficient amount of this compound is present for maximum growth, addition of any amount of sulphanilamide will not produce inhibition.

This has been explained on the basis that the only function of p-aminobenzoic acid in this organism is as a precursor for pteroylglutamic acid, and that sulphanilamide inhibits this conversion. When once enough pteroyl glutamic acid is added, the inhibited enzyme system is no longer necessary and growth proceeds normally.

In the case of *E. Coli* it is found that the inhibitory effect of sulphanilamide is overcome non-competitively to a certain extent by methionine (Winkler and de Haan 1948). But when the concentration of sulphanilamide exceeds a certain value, addition of large amounts of methionine will not reverse the toxicity. Methionine has the effect of raising up the inhibition index, as a larger amount of sulphanilamide will be required to produce the same inhibition in its presence than in its absence. If in the presence of methionine, xanthine is added to the above system, it is able to reverse the toxicity of sulphanilamide to a greater extent and raise the inhibition index still further. But the sulphanilamide-inhibition is not overcome completely. Also xanthine exerts its reversing effect only if methionine is already present in the medium. Similarly it is found that serine, pteroylglutamic acid and valine added in the above order reverse the effects of sulphanilamide non-competitively to an increasing extent as shown by the large increases in the inhibition index in the presence of these compounds.

All these observations have been explained on the assumption that these compounds, methionine, xanthine, serine, pteroylglutamic acid and valine are all products in the biosynthesis of which p-aminobenzoic acid participates. Sulphanilamide exerts its bacteriostatic effects through inhibition of the various enzymes participating in the synthesis of the above compounds. Also the inhibitor preferentially affects the various enzyme systems so that the one that is most affected constitutes the limiting reaction. In this case, the enzyme system primarily affected at low concentrations of sulphanilamide is the one leading to the production of methionine ; so that when once this is added, the inhibitory effect of sulphanilamide is overcome. But as this is not the only enzyme system affected, the reversal is not complete. With increasing concentrations of sulphanilamide, various other enzyme systems are affected so that the products of these enzymic reactions, i.e. xanthine, serine, pteroylglutamic acid and valine must also be added if the sulphanilamide toxicity is to be completely overcome.

Applications of Inhibition Analysis :

Since the antivitamins specifically inhibit the various metabolic functions of the vitamins and make possible a quick experimental production of extensive vitamin deficiencies, they offer themselves as powerful tools in biochemical investigations on vitamins. An analysis of the effects of an inhibitor and the counter-

action by the vitamin has led to great advances regarding the metabolic functions of various vitamins.

P-aminobenzoic acid itself was recognised as an essential metabolite for various organisms only when attempts were made to explain the bacteriostatic effects of the inhibitor sulphanilamide (Woods 1940). The bacteriostatic effect of sulphanilamide was then explained as due to competition with p-aminobenzoic acid, since this compound always counteracted the bacteriostasis due to the sulpha drug.

Inhibition analysis has shown that certain vitamins can take part in a number of metabolic reactions. Winkler and de Haan (1948), in the example already cited have shown that p-aminobenzoic acid functions in the synthesis of various metabolites like methionine, xanthine, pteroylglutamic acid, serine and valine.

The fundamental function of p-aminobenzoic acid and folic acid has also been revealed through the use of inhibitors. It was observed that an amine, 4-amino 5-imidazole carboxamide, accumulates in the medium when *E. Coli* is partially inhibited with sulphanilamide (Stetten and Fox 1945) and disappears when paraaminobenzoic acid is added to the medium. Evidently p-aminobenzoic acid is involved in the further metabolism of this amine and sulphanilamide inhibits this leading to an accumulation of the amine. The amine has previously been shown to replace purines in *Lactobacillus arabinosus* so that it evidently is a precursor of purines. Since other experiments have shown that p-aminobenzoic acid functions in the biosynthesis of purines (Snell and Mitchell 1942), it takes part presumably in the conversion of this amine into a purine. Since this conversion just entails the addition of a 'single carbon atom', the basic function of para-aminobenzoic acid is postulated as involved in metabolic reactions of "Single carbon units". The role of p-aminobenzoic acid in serine-glycine interconversion and also in the biosynthesis of methionine—probably from homocysteine by transmethylation—can also be explained on the above postulate.

Investigations of the bacteriostatic effects of pantoyl taurine on streptococci led McIlwain to conclude that pantoyl taurine inhibits the metabolism of pantothenic acid (McIlwain and Hughes 1944), and that normally pantothenic acid is conjugated into a larger and presumably more important substance. The conjugate mentioned can now be identified with coenzyme A.

Sivaramakrishnan and Sarma (1953) have studied the effects of sulphanilamide on amino acid changes during germination of green gram (*Phaeseolus mungo*), and the counteraction by the vitamins p-aminobenzoic acid and folic acid.[33] Sulphanilamide produces decrease in the levels of tyrosine, methionine and serine, and increases in histidine and arginine, while threonine remains unaffected. A direct metabolic relationship between histidine and methionine has been noted. P-aminobenzoic acid is directly concerned in histidine metabolism, folic acid takes part in tyrosine metabolism, both the vitamins participate in the metabolism of serine and methionine while neither of them seems to be involved in the metabolism of threonine.

Limitations of inhibition analysis:

Inhibition analysis suffers from certain limitations which must be clearly understood before it can be put to any good purpose. These limitations arise as a direct result of some of the properties of the inhibitors.

Some of the inhibitors are characterised by a high species specificity. While they act as inhibitors for some species, they actually have vitamin potencies in some others. For example desthiobiotin acts as an inhibitor of biotin in *Lactobacillus casei;* but it has biotin activity for *Saccharomyces cereviseae.* Methoxypyridoxine acts as an antivitamin for chicks and dogs, but is not an antivitamin for rats. Hence a careful choice of the antivitamin with particular reference to the organism studied should be made.

Also the inhibitor is much more specific than the metabolite in some cases. While the vitamin takes part in a number of enzymic reactions, the inhibitor affects only some of these enzyme systems. For example, while desoxypyridoxine inhibits the conversion of tryptophan into nicotinic acid, it has no effect on transamination (Schwartzmann & Hift 1951). The vitamin is known to take part in both these processes.

Another point to be noted is the occasional anamolous behaviour of some antivitamins. In one and the same organism these antivitamins act either as growth promoters or as inhibitors depending on their concentrations. Usually at low concentrations they exert stimulatory effects while at high concentrations they become inhibitors. Such anamolous behaviour has been noted with sulpha drugs (Green 1940), and pyrithiamine (Woolley & White 1943b). This essentially implies complete lack of co-

ordination and continuity between the concentration of the inhibitor and its effect. This is a drawback in those investigations where the effect of varying concentrations of an inhibitor on any particular metabolic process is studied.

Since the antivitamins interfere with the vital processes of life, they are toxic by nature. Some of them like aminopterin are so toxic that they produce quickly a widespread dislocation of the entire body metabolism. This seriously affects their use as inhibitors in metabolic studies.

To conclude, inhibition analysis is certainly a powerful tool and offers great scope in biochemical investigations. It has already yielded excellent results. But it is a double-edged tool, sharp at both the edges, and must be cautiously applied with a thorough knowledge of its various peculiarities.

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Seasonal changes in the Hydrogen-ion concentration and the dissolved Oxygen content of the surface waters of the Madras Coast*

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ABSTRACT

Measurements of temperature, salinity, pH and dissolved Oxygen in the surface waters of the Madras Coast were made during the period February 1951 to April 1952. The seasonal variations of pH dissolved Oxygen are discussed with reference to temperature, salinity and also diatom variation on the basis of mean monthly values.

pH is shown to be independent of changes in Oxygen content and is probably dependent on the carbon-dioxide content which is affected by photosynthesis. The Oxygen content is found to be influenced by water temperature rather than by photosynthetic activity.

INTRODUCTION

Inasmuch as the ultimate source of food for all life in the sea depends on the photosynthetic activity, changes in hydrogen-ion concentration and Oxygen content of the surface waters are of biological importance and afford a measure of the intensity of life. Sea water gains its dissolved Oxygen from the atmosphere direct, as well as from the Oxygen set free during carbon-assimilation. The changes in the Oxygen content are more easily appreciable than the fluctuations in carbon-dioxide which are easily masked due to the buffering action of the bicarbonates in sea water. The pH value, dependent as it is, on carbon-dioxide content, does not alter so readily as the Oxygen content. The carbon-dioxide lost in the process of photosynthesis is again gained due to the hydrolysis of bicarbonates, respiration of organisms as well as due to the tendency for carbon-dioxide of surface water to be in equilibrium with that in the air.

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Moore et al (1915), Atkins (1922), Marshall and Orr (1930) and Cooper (1933) used these changes in pH and Oxygen content as indices of organic production in the sea, though the values obtained can only be of a minimal nature, because of a number of disturbing factors like consumption of Oxygen by animals, plants and bacteria and interchange with atmosphere and different water masses (Ketchum 1951). Compared to what has been done on the seasonal changes in pH and Oxygen in the high latitudes, the information available from the tropics is very scarce and is mainly due to the contributions of Orr (1933) in the Great Barrier Reef region which showed that the pH values, Oxygen content as well as the diatom growth were uniform and not marked by seasonal differences. Observations by Riley (1939) in the Western North Atlantic, were too limited in period and those by Smith et al (1950) in the inshore waters adjacent to Miami, were too few in number, to permit any generalisations. Our knowledge of these biological factors in the Indian waters appear equally incomplete. Thompson and Gilson (1937), Mohamed (1940), Chidambaram et al (1951) and Raghuprasad (1952) collected data from the observations extending over short periods in the Indian seas. Bal et al (1946) recorded the pH variations of Bombay Harbour waters and Jayaraman (1951), the dissolved Oxygen content of Madras inshore waters, without reference to the factors behind the pH and Oxygen content fluctuations. The present work is attempted in the hope that a study of the factors known to effect the fluctuations in pH and Oxygen content will add to our knowledge of the hydrography of the sea close to Madras.

Material and Methods:—The results to be recorded in this paper are based on the analyses of a number of sea water samples taken from the surface waters of Madras Coast during the period February 1951 to April 1952. The observations were restricted to a single inshore area, east of the Laboratory. Water samples were brought to the Laboratory in coloured bottles which were completely filled, leaving no air space over the water. By this, the changes that may take place in sea water due to evaporation and gaseous exchange can be avoided. The analyses were done within a few hours after collection. Dissolved Oxygen was determined by Winkler's titration method and the percentage saturation was calculated by the aid of the Fox's formula. pH was estimated on the Hellige's comparator, using Cresol Red as indicator and the values were corrected for salt error. Salinity was determined by Mohr's method of titration of chlorides, using

silver nitrate. The results are discussed on the basis of mean monthly values.

Results and Remarks:—The results of observations on pH, dissolved Oxygen, percentage saturation, temperature and salinity of sea water are given as monthly averages in the table that follows and the mean monthly variations in pH, dissolved Oxygen, temperature and salinity are plotted in Figs. 1 and 2.

TABLE

Months	Temperature °C	Salinity %	pH	Dissolved Oxygen ml/L	Percentage saturation
1951					
February ..	27·30 (9)	30·63 (9)	8·16 (9)	4·055 (4)	83·37
March ..	28·60 (11)	34·52 (12)	8·10 (12)	3·925 (5)	84·72
April ..	29·30 (6)	34·94 (9)	8·17 (9)	3·920 (3)	85·96
May ..	29·20 (3)	34·68 (3)	8·20 (2)	3·940 (3)	86·08
June ..	28·00 (2)	34·32 (2)	8·15 (2)	4·105 (2)	87·40
July ..	28·30 (9)	34·44 (8)	8·00 (8)	4·018 (4)	86·18
August ..	28·00 (11)	34·12 (13)	8·02 (13)	3·940 (4)	83·90
Sept. ..	29·40 (9)	33·53 (9)	8·02 (10)	3·936 (5)	85·65
Oct. ..	30·10 (7)	32·39 (10)	8·05 (10)	3·815 (4)	83·50
Nov. ..	28·50 (7)	26·24 (10)	8·11 (9)	4·030 (4)	84·43
Dec. ..	27·40 (6)	28·80 (6)	8·12 (6)	4·293 (3)	87·36
1952					
January ..	27·16 (5)	30·06 (7)	8·07 (7)	4·373 (3)	89·35
February ..	27·80 (4)	30·73 (6)	8·17 (6)	4·183 (3)	86·80
March ..	28·48 (4)	33·69 (7)	8·18 (6)	3·947 (4)	84·55
April ..	30·07 (6)	33·84 (8)	8·24 (9)	3·955 (4)	87·32

(Figures in brackets denote the number of observations made in each month, from which the average is computed.)

The average values for pH ranged from 8·00 to 8·24 (Fig. 1) and did not follow the salinity changes. They were high during the period April-June. In July there was a fall with an increase in the subsequent months till December. In January again there was a slight fall and in the period that followed pH was rising. The values stated are only approximate and it is fully realised that accurate variations in pH could be followed only with more sensitive methods of pH determination.

The mean Oxygen content ranged from 3·815 ml/L. to 4·373 ml/L. (Fig. 1). The values are very low compared to those of temperate waters. Maximum values were noticed during the period November-January, which is the cold season for Madras.

The Oxygen content throughout the period was never very far from saturated. This is in support of the observations made by

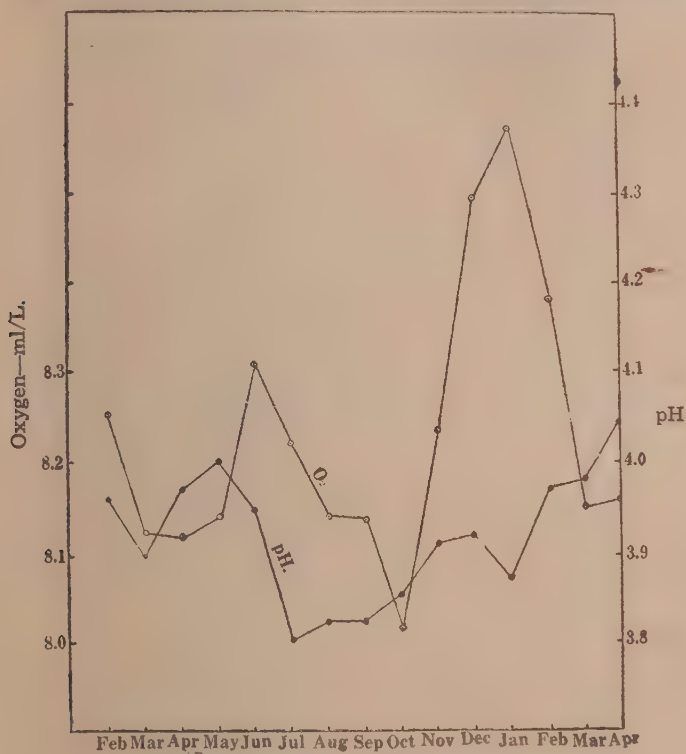

FIG. 1: SEASONAL VARIATIONS IN pH AND DISSOLVED OXYGEN

Thompson & Gilson (1937), Riley (1939), and Smith et al (1950) who showed that supersaturation is not common in tropical waters. They showed that maximum values occurred at a few metres below the surface, which is the zone of maximum photosynthetic activity in the tropics. Orr (1933) discussing the reasons for such undersaturation opined that the respiratory activity of animals which is at a higher rate in tropics due to higher temperature as well as the oxidation of the dead organic matter in the sea-water may be responsible for the undersaturation. The seasonal changes in pH and dissolved Oxygen were not so well pronounced as in temperate latitudes. Orr (1933) who noticed negligible seasonal changes in pH and dissolved Oxygen in the Great Barrier Reef region attributed them to the absence of any marked seasonal cycle in diatom growth.

The average monthly temperature showed a range from 27·16°C to 30·1°C (Fig. 2). It was found to be rising for the period February-April and again during September-October. It was low through November to January.

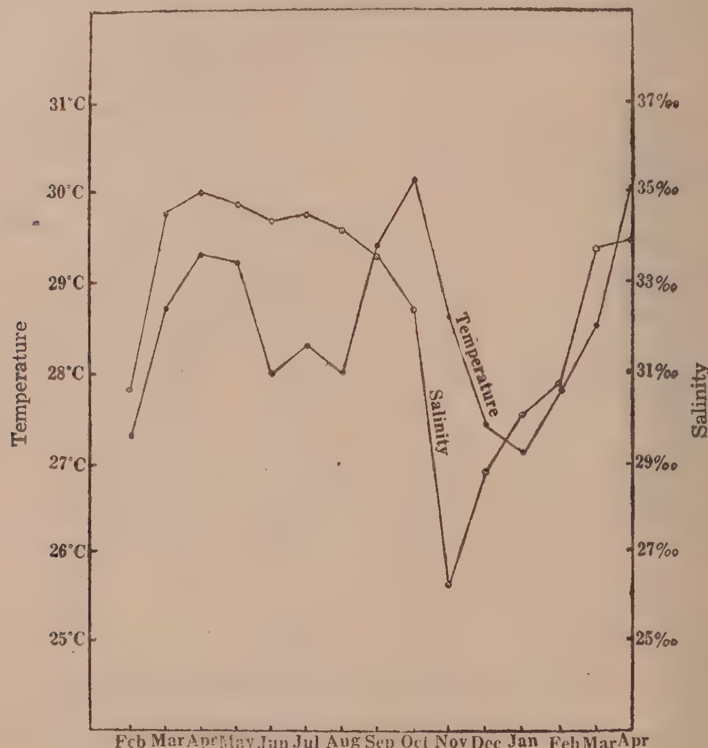

FIG. 2: MONTHLY AVERAGE TEMPERATURE AND SALINITY RECORDS

It will be seen that changes in pH do not show any definite relation to changes in dissolved Oxygen. A quantitative study of the diatoms from 135 plankton collections made during the period February 1951—April 1952 showed that the distribution of the diatoms was not well marked by any seasonal cycle. Diatom peaks were noticed in the months of April, May, August, September, November and December in 1951 and February and April in 1952. It is clear that the pH, though it does not follow closely changes in phytoplankton, increases in the months when the diatom peaks occur. Herdman (1918) and Hutchinson et al (1929) attributed the high pH which coincided with the diatom abundance to the utilisation of carbon-dioxide by phytoplankton. Similarly the results obtained by Marshall and Orr (1927, '30), Cooper

(1933) and Kokubo and Tamura (1938) which showed parallel changes between pH and diatoms, were in general agreement with the seasonal changes already found by Atkins (1922-24).

It is evident that in the Madras coastal waters, pH is affected by changes other than Oxygen. It is probably dependent on the total carbon-dioxide content and its partial pressure which is affected by biological activity. pH rises due to the utilisation of carbon-dioxide by phytoplankton and the Oxygen liberated during photosynthesis is lost to the atmosphere due to high temperature. McClendon (1916) observed that contrasted with the behaviour of Oxygen, no certain effect of the temperature on carbon-dioxide content of water, as indicated by pH, was noticed. Moore et al (1915) attributed the diatom increase to the variations in the alkalinity of the sea water which depends upon the amount of carbon-dioxide held in solution. Menon (1931) discussing this factor stated that variations in alkalinity are hardly so much the cause as the effect.

The fluctuations in the surface values for the dissolved Oxygen are found to be mainly a function of the variations in water temperature, since the minimum value in October and the maximum value in January coincided closely with the maximum and minimum of water temperature. The increase in Oxygen content during the period November-January takes places at a time when the salinity is also low and this may be attributed to the increased solubility of Oxygen in sea-water due to low salinity besides to the decreasing temperature. The slight increase in the Oxygen value noticed in April 1952, in spite of the increase in temperature, may be ascribed to the high photosynthetic activity, as was evident from the diatom blooms noticed in that month. It is curious to note that for the first six months of observation, Oxygen curve shows an inverse relationship to the temperature and salinity curves, which is due to the variations in the solubility of Oxygen caused by changes in temperature and salinity. However, since it is with the temperature curve that Oxygen shows close relation throughout, it can be said that the Oxygen content of the surface waters here is almost exclusively determined by water temperature and the effect of photosynthesis is not conspicuous. It may be mentioned here that Jayaraman (1951) who made a seasonal study of the Oxygen gas content of the surface waters of the Madras coast suggested that the variations in the dissolved Oxygen might possibly be due to the effect of photosynthetic activity, which is in support of the observations made by Marshall and Orr (1927,

'30) and Riley (1941). Cooper (1933) noticed a rapid increase of Oxygen with the diatom outburst when the temperature also was low. But with rising temperature, the degree of supersaturation fell due to the loss of Oxygen to the atmosphere. The results obtained in the present study are in conformity with the observations made by Uda (1929), Ito (1930), Dakin and Colefax (1935) and Kokubo and Tamura (1938). Redfield (1948) while determining the magnitude of the exchange of Oxygen across the sea surface in the Gulf of Maine, found that Oxygen left the surface during the summer months, due to decreasing solubility, resulting from the warming of water and re-entered during the winter months due to surface cooling.

Acknowledgements

I take this opportunity to express my grateful thanks to Dr. C. P. Gnanamuthu, Director, University Zoology Laboratory, for his help and guidance in the preparation of this paper and to Dr. G. Krishnan, Reader, University Zoology Laboratory, for his helpful criticisms in the course of this work. I am also thankful to the Syndicate of the Madras University for the award of a studentship, during the tenure of which the present work was carried out.

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Pelagic Copepoda of the Madras Coast*

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ABSTRACT

In the course of a detailed study of over 250 samples of plankton collected at Madras as well as Krusadai Is., 69 species of Calanoids, 21 species of Harpacticoids, 26 species of Cyclopoids, 1 Monstrilloid and 2 Caligoids were identified. Of these 39 are being recorded for the first time from Madras. These are being dealt with in three parts—Part I—Cyclopida, and Part II Calanoida will appear in this journal. The third part dealing with Harpacticoida is being published elsewhere.

INTRODUCTION

Historical: Our knowledge of the Copepods of the Indian and the neighbouring seas is derived from the works of only a few. Of these, the earliest is a paper by Giesbrecht (1899) which was followed by Cleve (1901, 1904), Wolfenden (1906) and Scott (1909). The foundation of our knowledge of the copepods of the Bay of Bengal was laid by Thompson (1899) who gave an account of the copepods collected by "S. S. Johannesberg". In recent years (1914-1949), the copepods of the Bay of Bengal (1919, 1929, 1932, 1940, 1947 and 1949), the Gulf of Manaar (1914) and the Arabian Sea (1940, 1947, 1949) have been studied exhaustively by Sewell. The copepods of the southern region of the Madras Coast, that is, the Gulf of Manaar are included in Thompson and Scott's work (1903) on the copepods of the Ceylon Pearl Banks. Hornell and Ramaswamy Naidu (1927) recorded a few common forms in their plankton report of the Sea off Calicut on the West Coast of India. Copepods of the Madras waters are known from a paper by Menon (1930) who recorded forty-five forms in his account of the Madras plankton. Gopala Iyer, Menon and Menon (1934) have included these common forms in their plankton calendar for 1929-30. Menon (1945) has recorded forty three

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forms in his paper on the plankton of the Travancore Coast and Jacob and Devidoss Menon (1947) have dealt with the seasonal distribution of 34 common forms off Calicut. Recently Chacko (1950) has included twelve forms in his account of the plankton of Krusadai Islands and the writer has described 7 species (1951) from the Madras coast.

Confined as it is to the above studies, our knowledge of the copepods, particularly of the Madras Coast, is very meagre, when compared with the exhaustive accounts of the copepod fauna of other countries. There is no complete account of the copepods of any one locality. Hence, a detailed study of the numerous species, genera and families of the truly pelagic copepods was considered useful in the light of their importance in Fisheries and the Biology of the Indian Seas in general.

The area studied: The present paper includes a study of all the copepods in the plankton collected at Madras inshore water, Madras harbour, Cooum and Adyar backwaters, the inshore and off shore plankton collected around Krusadai Islands.

In the present study 113 species and varieties of copepods have been identified and described. These will be dealt with in three parts.

Material and Methods: About 260 preserved as well as fresh samples were examined. Collections made in the Zoology Laboratory, Madras, from 1945-47 and preserved in separate dated bottles, were studied in addition to fresh daily collections made from July 1947-49. The older collections were used to corroborate as well as to complete the weekly tally, especially on certain days when fresh collection was not possible due to rough weather. The plankton collected from the environs of Krusadi Island, collected, dated and preserved by the Madras Fisheries Department, during a period of ten years were available. Of these the collections made from 1936-38 were examined together with the daily plankton reports; where there happened to be gaps of a few days, the previous year's collections came handy. The author takes this opportunity to express his thanks to the Deputy Director of Madras Fisheries for all help. In order to secure information about colouration and other details which might be lacking in the preserved collections, the author visited the area and townetted around the Island and off Rameswaram Coast both at night and break of day.

All the drawings were made with the aid of Camera lucida. Where less than 20 copepods have been taken, their numbers are given. The measurements given in this account do not include the furcal setae unless otherwise seated.

The author wishes to record his grateful thanks to Prof. C. P. Gnanamuthu, Director, Zoology Laboratory, Madras University for guidance and help and Col. R. B. Seymour Sewell, F. R. S. of Cambridge for several valuable suggestions and helpful criticisms. To the Syndicate of the Madras University the author is very thankful for the award of a studentship during the tenure of which the present work was done.

SUB-ORDER: *CYCLOPOIDA.*
Family: OITHONIDAE.
Genus: *Oithona* Baird.
Oithona lineuris Giesbrecht.
(Wolfenden, 1906. Pl. XCIX, Fig. 49).

Occurrence: Several females of this copepod were taken in the plankton collected in July and January from Madras. This is the first record of the occurrence of this copepod at Madras. This *Oithona* is known to occur in Red sea, Ceylon Pearl Banks, and Maldives.

Size: Length female 1·1 mm.
Colour: Transparent and colourless.

Salient features: As the name suggests the body is long and slender. The rostrum is not visible on the dorsal side and is bent on the ventral side. The ratio between the length and breadth of the cephalothorax is 3:1. The formula of the outer edge spine on the first four pairs of swimming feet is 1.1.3, 1.1.2, 1.0.1, 0.0.1. The spinulation on the feet are very fine.

Oithona robusta Giesbrecht (Fig. 1).
(Rosenderon, 1917, p. 29-32. Figs. 166-c).

Occurrence: This is the first record of the occurrence of this copepod at Madras. Seven females of this Oithonid were present in the plankton collected at the Madras Harbour on July 23rd and 26th, 1948.

Distribution: Mediterranean, Laccadives and Maldives, Indian, Atlantic and Pacific oceans, and the Arabian sea.

Size: Length 1.5 mm.

Colour: Transparent and colourless. The plumose setae in the mandibular palp are coloured red.

Salient features: The animal has a very robust appearance as the name suggests. The rostrum is pointed and directed forwards. The setae in the palp of the mandible are long and plumose. The exopods of first three pairs of swimming feet have 1, 1, 3 outer spines. The fourth feet has only 1, 1, 2 outer spines.

Oithona setigera Dana.
(Rosenderon, 1917, p. 20-24, Figs. 10 a—11 b).

Occurrence: This is the first record of the occurrence of this copepod at Madras. Several females were collected at Madras between July and September.

Distribution: Pacific, Mediterranean, Maldives and Laccadives, Gulf of Guinea, Indian Ocean, Nicobar Islands.

Size: Length 1·3 mm.

Colour: Colourless and transparent except for the red colour of the 'clavate' setae on the second basals.

Salient features: Rostrum pointed and directed anteriorly, antennule reaching upto the caudal furca. The second basals of the swimming feet bear a 'clavate' seta laterally (Fig. 2). Exopodites of first three pairs of swimming feet have 1, 1, 3 outer spines. The fourth swimming feet has 1, 1, 2 outer spines. Fifth leg is represented by two long plumose setae.

Oithona plumifera Baird.

(Giesbrecht 1892, P. 537, Taf. 4, figs. 10; Taf. figs. 12, 13, 22, 25, 27, 28, 32, 33, 44-47; Taf. 44, figs. 1, 7, 12-15).

Occurrence: This copepod occurs in July and January at Madras but never in large numbers.

Distribution: Almost worldwide in distribution.
Size: Length 0·93 mm.

Salient features: The animal is slender and long. Basipodite of legs 2 to 4 carry plumose setae. The exopodite of the mandibular palp bears only four setae (Fig. 3). The setae in the endopodite are plumose. The abdominal segments are fringed with spinules on their posterior margin. The inner side of furca hirsute (Fig. 4).

Oithona challengeri ? Brady.
(Brady 1883, p. 97, Pl. XL. Figs. 1-10).

Occurrence: This copepod occurs in July and January. Several females have been taken.

Size: Female 1·4 mm.

Colour: Transparent and colourless. The outer setae on the caudal furca and the furcal joint itself light violitish red.

Salient features: Cephalothorax narrow, tapered towards the anterior end. Antennule very long, reaching upto the anal segment. The setae on the basipods of legs 2 to 4 not plumose. The setae on the first mandibular lobe scarcely plumose. The exopodite carries five plumose setae. (Fig. 5). The two lateral setae in the furcal rami are plumose. (Fig. 6).

Oithona rigida Giesbrecht.
(Giesbrecht, 1898, p. 324. Fig. 10-15).

Occurrence: In the Madras as well as Krusadai plankton large numbers of both males and females are found throughout the year.

Size: Female 0·65 mm., male 0·5 mm.
Colour: Of a light dirty green.

Salient features: The cephalothorax is ovate. The anterior end of a cephalosome squared. Has a very rigid appearance as the name suggests. The outer edge spine formula for the first leg is 1. 1. 3. Fifth leg rudimentary represented by a small knob-like projection tipped with two setae.

Occurrence: This copepod is present throughout the year in the area under investigation. This appears to breed in July and January in Madras waters.

Family: CLAUSIDIIDAE.
Genus: *Saphirella* T. Scott.
Saphirella enigmatus Krishnaswamy.

(Krishnaswamy, 1952. 8 P. 33. Text Fig. 5).

Occurrence: Over 20 specimens were collected from Madras as well as Krusadai between July and March.

Colour : The animal is of a dark yellow colour and has a very robust appearance.

Salient features: Body very robust. Length 1·5 mm. to 1·8 mm. Antennule five jointed. The outer edge of the terminal joint of the *antenna* serrate. The endopodite of swimming feet with one apical dagger-shaped spine and five sub-apical setae.

Genus: *Hemicyclops* Boeck.
Hemicyclops indicus Sewell.
(Sewell 1949. p. 69. Text. fig. 16).

Occurrence: A single female was found on 8-3-47 at Madras.

Distribution: Nicobar Islands.

Size: Female 1·04 mm.

Salient features: Cephalothorax longer than wide. Abdomen four jointed. Caudal rami longer than broad. Maxillipad two jointed, second joint very swollen and carrying a scarcely plumose seta about its middle and two stout claw-like spines terminally. Endopods of swimming feet longer than exopods. The coxopodite as well as the basipodite of first leg carries a plumose seta. Fifth leg broad and lamellar, with outer and inner margin armed with teeth and carrying three spines.

Family: CLAUSIDIIDAE.
GENUS: *Hippomolgus* Sars.
Hippomolgus dubia (Thompson and Scott), (Fig. 7).

(*Hersiliodes dubia*, Thompson & Scott, 1903, p. 284. Pl. III. Figs. 18-27).

Occurrence: This is the first record of this copepod from Madras and has been previously known from Suez Canal. Thompson and Scott established this from a single male and the author agrees with Nicholls (P. 46, 1944) that this "copepod is clearly a *Hippomolgus* the only male so far described for this genus". Two males were found in the plankton collected at Madras on 12-3-49.

Size: Male 1·8 mm.

Colour: Of a uniform, dirty, light brown colour.

Salient features: Cephalothorax longer than broad and the anterior and is produced into a knoblike projection. The antennule is short and compact. Genital segment nearly as broad as long. Abdomen five jointed. Fifth leg consists of a quaduate joint with two apical and one sub-apical spine. (Fig. 8).

Remarks: The Madras specimens differ from Thompson and Scott's in the absence of a seta near the spine towards the outerside on the fifth leg.

Family: LICHOMOLGIDAE.
Sub-family: Lichomolginae (Gurney).
Genus: *Kelleria* Gurney.
Kelleria rubimaculata Krishnaswamy.

(Krishnaswamy: 1952, P. 326. Text fig. 3).

Occurrence: Over fifty females and males of this form were collected between July and March.

Salient features: Length 0·7 mm. female and 0·637 mm. male. *Antennule* seven jointed. The third joint of the *antenna* armed with two setae and a claw-like spines. The ventral edge of *mandible* hirsute towards the basal sides. *First Maxilla* consists of a simple lappet with three spines. *Maxilliped* is three jointed in the female. The spine on the distal end of second joint not bifid. *First swimming feet*: Basipod without an outer seta. Third exopod joint with five lateral spines. *Second swimming feet*: Third exopod joint with three serrate spines and naked spines. Endopod of third joint with one spine. Basipod of fourth leg with an outer seta and three inner spines.

Genus: *Macrochiron* Brady.

Sub-genus: *Paramacrochiron* Sewell.

The sub-genus *Paramacrochiron* was created by Sewell in 1949 to accommodate the forms "in which the endopod of fourth leg is composed of only a single segment. These species were previously included in the genus *Pseudanthessius* Claus, but the presence of a well developed 5th leg necessitates their removal from that genus" (P. 108 Sewell) (Loc. cit.). The following species have been transferred to this genus by Sewell *P. maximum, P. chelifer, P. parvas* and *P. fusciolus* T. Scott. Sewell has added a new sp. from Andaman Islands, *P. malayense*. A new species, *P. ornatus* has been described from Madras by the writer (1952).

Macrochiron (*Paramacrochiron*) *ornatus* Krishnaswamy.
(Krishnaswamy 1952. Text fig. 4).

Occurrence: Large numbers of this form were collected between August and March in the plankton.

Size: Length 0·84 female 0·635 male.

Colour: When alive, the animal is of a dirty yellow colour with a number of blue patches on the dorsal side of the body and even on the swimming feet. These blue patches fade away after preservation in formalin.

Macrochiron (*paramacrochiron*) *maximum*. (Thompson & Scott) (Fig. 9).
(*Pseudanthessius maximus* Thompson and Scott, 1903) (p. 276. Pl. XIV. Figs. 1-11).
(Sewell 1949. p. 108-109).

Occurrence: This is the first record of the occurrence of this copepod at Madras. About twenty females and five males were taken in the plankton collected on the 3rd March 1949.

Distribution: Previously known from Ceylon Pearl Banks, and Andaman Islands.

Size: Length female 3·4 mm. Male 2·5 mm.

Colour: Transparent and of a slight rose colour.

Salient features: Cephalosome anteriorly rounded. Body very robust. The terminal joint of the antenna carries a stout spine and three setae. Fifth leg well developed and tipped with two setae.

Family: ONCAEIDAE.

Genus: *Oncaea* Philippi.

Oncaea venusta philippi.

(Wilson 1932 a. P. 353-54 Fig. 213 a-d).

Occurrence: This copepod which occurs at Madras in November and December in large numbers, enjoys a wide distribution.

Size: Female 1·0 mm. Male 0·8 mm.

Colour: Body is of light orange colour with scattered carmine spots. Genital segment is deep carmine.

Salient features: Cephalothorax obovate. Abdomen half as long as the cephalothorax. Fifth leg in female small, tipped with two sub-equal setae. The genital segment is very swollen in the male.

Remarks: This copepod appears to breed in November and December when they are usually seen in copula.

Oncaea conifera Giesbrecht.

(Giesbrecht, 1892; Vol. 19, p. 591. pl. 2, 47).

Occurrence: This copepod which has been recorded from almost all the great oceans is present in the Madras plankton in August and September and August, September and November in Kursadai plankton, widely distributed.

Size: 1·1 mm. Female; Male 0·8 mm.

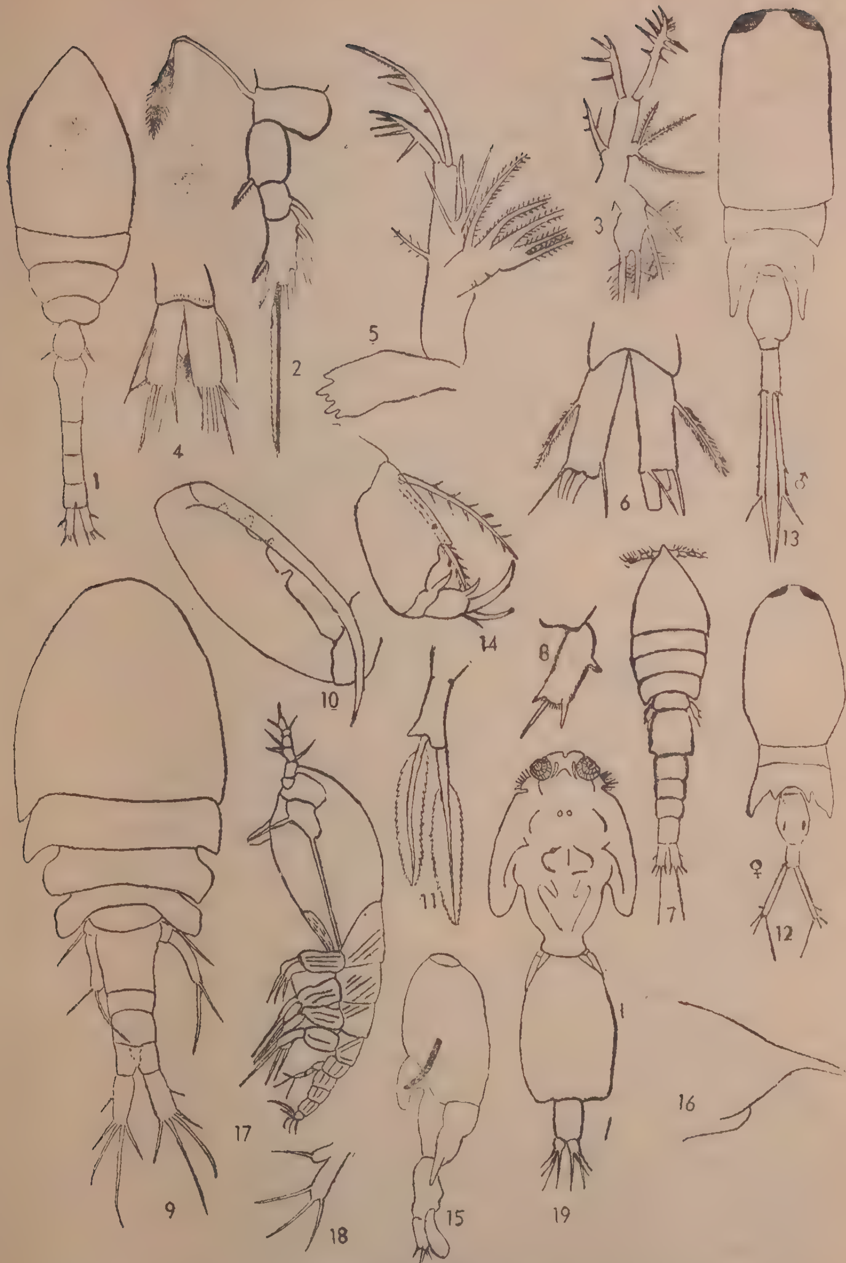
Colour: The whole body is coloured deep yellowish orange the entire sides are deep orange in colour. Red pigment spots are found scattered on the cephalothorax.

Salient features: Body pyriform. Abdomen one third the length of the cephalothorax. Genital segment twice as long as the abdomen. Furcal rami as long as the anal segment. Fifth leg in female tipped with two unequal setae. The genital lappets large and conspicuous in the male and abdomen is five jointed.

Remarks: The present form agrees with type (a) of Farran (1936), like Sewell's specimens from Nankauri Harbour and Arabian sea.

Genus: *Lubbockia* Claus.

Lubbockia sqillamana claus (Figs. 10, 11).



TEXT-FIGURES 1-19

TEXT-FIG. 1. *Oithona robusta,* ♀, dorsal view (× 56); 2. *O setigera* second basal, (× 280); 3. *O. challengeri?* mandible (× 280); 4 furcal rami (× 280); 5. *O. plumifera* mandible, (× 280); 6. furcal rami (× 280); 7. *Hippomolgus dubia* ♂ (× 280); 8. 5th leg, (× 400); 9. *Macrochiron (paramacrochiron) maximum, dorsal view* ♀ (× 40); 10. *Lubbockia squillamana,* maxilliped, (× 280); 11. 5th leg. (× 400); 12. *Corycaeus (Corycaeus) speciosus* ♀ (× 56); 13. ♂ (× 80); 14. *Corycaeus (Agetus) elongatus* antenua (immature stage (× 400); 15. *Corycella gibbula* ♀ (× 80); 16. Posterior thoracic margin (× 280); 17. *Monstrilla sp.* ♀ (× 56); 18. 5th leg. (× 280); 19. *Caligus* sp. (× 56).

(Brady 1883, p. 118, Pl. LIII. Figs. 12-16; Pl. LIV. Figs. 1-17).

Occurrence: This is the first record of the occurrence of this copepod at Madras. Four females of this were found in the plankton collected at the Madras harbour on 23-7-1948.

Size: 2 mm. long.

Colour: Transparent and colourless. Red pigment is scattered about on the cephalothorax.

Salient features: Cephalothorax elliptical in shape. The first cephalothoracic segment twice as long as the succeeding segment. Antennule very short, scarcely half as long as the cephalosome. Maxilliped well developed and prehensile (Fig. 9). Abdomen four jointed. The genital segment is slightly dilated towards the anterior end. Fifth leg is single jointed and is tipped with two folisceous setae (Fig. 10).

Family: CORYCAEIDAE.
Genus: *Corycaeus* Dana.
Sub-genus: *Corycaeus* Dahl.

Corycaeus (*Corycaeus*) *speciosus Dana* (Figs. 12 and 13).

(Wilson, 1932 a—p. 358, Fig. 21 a and b).

Occurrence: This is the first record of the occurrence of this copepod at Madras. Several males and females of this were present in the Madras plankton from September to November.

Distribution: Tropical Atlantic, Canary Isles, Pacific, Red sea, Indian ocean and Arabian sea, Mediterranean, Chesapeake Bay, Woods Hole.

Size: Female 1·6 mm. Male 0·8 mm.

Colour: Of a light yellowish colour. Eye red.

Salient features: The anterior end of the cephalosome rounded. Third and fourth thoracic segment fused. The third segment produced into acute processes. Genital segment swollen in the middle. Caudal ramii long, longer than the combined length of genital and the abdominal segment and divergent. Antenna with a powerful claw. The basal joint carries two unequal spines. The second joint carries two long claws and two short claws. Endopod of fourth swimming feet consists of a short knob with a seta. The body is narrow and slender in the male. Genital segment swollen and carries two spines posteriorly. Caudal ramus nearly one and half times longer than the genital and the abdominal segments together.

Sub-genus: *Agetus* Kroyer.

Corycaeus (Agetus) elongatus Dana.

(Wilson, 1932 a. p. 355-356. Fig. 214 a to c).

Occurrence: Several females and immature stages of this copepod were taken from the plankton from July to November.

Distribution: Gulf Stream, Malay Archepelago, Messina, Atlantic, Mediterranean, Red sea, Indian Ocean, Adriatic, Chesapeake Bay, Woods Hole and Madras.

Size: Length 1·3 mm. female.

Salient features: The anterior end of cephalosome rounded. The lappets on the fourth segment reaching to the middle of the genital segment. Abdomen single jointed, and slightly dilated towards the middle. Furca long, nearly as long as the genital segment with three apical and one sup-apical seta. The endopod of fourth swimming feet represented by a knob-like projection with a plumose seta.

Remarks: The immature stages are very common in September. The setae in the antennae are feathered as shown in the figure (Fig. 14). The feathered setae are considered to be characteristic of immaturity. (vide Farran p. 283, 1913).

Sub-genus: *Onychocorycaeus* M. Dahl.

Corycaeus (*Onychocorycaeus*) *giesbrechti* F. Dahl.

(Giesbrecht 1892, p. 659. Taf. 4. Fig. 12, Taf. 5. Figs. 22-34, 47).

Occurrence: This copepod occurs from July to February but never in very large numbers, both in Madras and Krusadai plankton.

Distribution: This copepod is known to occur in Tropical Atlantic, Tropical Pacific, California, Coast, Red Sea, Indian ocean, Gulf of Guinea, Travancore coast, Madras, Krusadai Islands.

Size: Female 1·3 mm. Male 0·95 mm.

Colour: Of a light yellow colour. Eye deep blue.

Salient features: Anterior end of cephalosome rounded. The cephalothorax is twice as long as the abdomen. 'Lappets' of the third segment scarcely reaching the middle of the genital segment. Genital segment barrel shaped. Caudal rami as long as the abdomen. The male is slightly smaller than the female and slender. The genital segment is longer than the abdominal segment. The anterior end of cephalosome appears truncate.

Corycaeus (*onychocorycaeus*) *ovalis* Claus.

(Dahl, M. 1912, p. 10-97. Taf. XIII. Figs. 9 to 16)

Occurrence: This is one of the common forms present in the plankton from September to April in Madras as well as Krusadai plankton.

Distribution: Tropical Atlantic, Pacific, Phillipines, Canary Islands, Malta, Tropical Atlantic, Mediterranean, Arabian sea, Indian ocean, Malay Archepelago, Madras, Red Sea, Adriatic.

Size: Female 1 mm. Male 0·85 mm.

Colour: Of a light yellow with red pigment scattered about. The optic cups are deep blue in colour.

Salient features: Anterior end of cephalosome rounded. The lappets in the thoracic segments three and four produced. Genital segment dilated towards the anterior side. Abdominal joint only 1/3 the length of the genital segment. Furca broader than long and carries three apical and one sub-apical seta. Body narrow in male, the cephalosome appearing 'squared' anteriorly. The genital segment is swollen and rounded.

Sub-genus: *Corycella* Farran 1911.

Corycaeus (*Corycella*) *gibbula* Farran (Fig. 15 and 16).

(Dahl. M. 1912, p. 115-118. Taf. XV. Fig. 1-4).

Occurrence: This is the first record of the occurrence of this copepod at Madras. This copepod is common from September to November in the Madras Plankton.

Distribution: Mediterranean, Indian ocean, Maldives and Laccadives, Christmas Island, Ceylon Pearl Banks.

Colour: Of a light bluish colour towards the sides of the cephalothorax, becoming lighter towards the middle. Yellow pigment is found scattered about on the cephalothorax. Male of a uniform light rose colour.

Size: Female 1 mm. Male 0·9 mm.

Salient features: The cephalosome with parallel sides and the anterior end with two prominent rostral lens. The thoracic segment indistinctly showing the division. The 'lappets' of the third thoracic segment produced acutely. There is a small blunt process at the base of it. (Fig. 16). The ventral 'beak' prominent and bent posteriorly. The genital segment is swollen in the middle and its dorsal surface is slightly rugged. Furca nearly half as long as the genital segment. The spines in the second antenna are 'feathered'. In the male the cephalosome is narrow. The 'lappets' of the third thoracic segment acute and reaching as far as the middle of the genital segment. The maxilliped has a long, powerful claw.

Genus: *Corissa* Farran.

Corissa Indica Krishnaswamy.

(Krishnaswamy, 1952. p. 323. Text Fig. 3).

In the townet collections made at the Madras Harbour on 23rd and 26th July 1948, six females and two males of the present species were found.

Size: Length 0·742 mm. ♀. 0·711 ♂.

Colour: The body is transparent and colourless. The optic cup is deep orange in colour.

Family: SAPPHIRINIDAE.

Genus: *Sapphirina* J. V. Thompson.

Sapphirina ovato-lanceolata Dana.

(Giesbrecht. 1892. p. 618. Pls. 1, 52-54).

Occurrence: This "by far the most gorgeous tinted copepod in the entire collection", has been taken in all months of the year in the Madras plankton. This copepod has a wide range distribution.

Size: Female 2·6 mm. Male 3·5 mm.

Colour: The male is brilliantly iridescent. The anal segment and the caudal ramii are colourless or slightly reddish.

Salient features: The cephalosome is broader than long in the female the broadest part being in the middle. Abdomen narrower than the cephalothorax. The endopod of fourth leg is longer than the exopod. The cephalosome is wider than long in the male, with the anterior and narrowed. Corneal lens on the ventral side.

Sapphirina metallina Dana.

(Lehnhofer 1929, pp. 284, 325. Figs. 16-18, 57, 58).

Occurrence: This is the first record of the occurrence of this copepod at Madras. (Two males were found in the collection made in September 1948). This form has a vide distribution.

Size: 2·8 mm.

Colour: Brilliantly iridescent. There is more yellow than in *S. ovato-lanceolata.*

Salient features: Body long and narrow. Corneal lenses are found at the anterior end. Cephalosome rounded on the anterior end. Fifth leg represented by a short joint tipped with two setae.

Sapphirina nigromaculata Claus.

(Lehnhofer 1929. pp. 304, 336. Figs. 38-41, 65, 66).

(Wilson, 1932 a. p. 372-373. Fig. 228. a to d.)

Occurrence: This copepod which has a wide distribution has been taken in the months of November and December in the Madras plankton.

Salient features: Cephalosome very wide. The first and second thoracic segment as wide as the cephalosome. The succeeding thoracic segments decrease in breadth gradually. Abdomen is narrow. The third segment of the antenna very short. Endopod

of fourth leg short. In the male the abdomen is broad giving an oval outline to the body. The corneal lenses visible on the dorsal side.

Genus: *Copilia* Dana.

Copilia mirabilis Dana

(Karl Lehnhofer 1926, p. 136, Fig. 13, 1-5).

Occurrence: This copepod which is claimed by Farran (p. 114, 1936) to be the commonest species of Copilia in the Great Barrier Reef is being recorded for the first time at Madras. This copepod is present in the plankton from September to November but never in large numbers and is known to occur in all the great oceans of the world.

Size: Female 3·2 mm. Male 4·5 mm.

Colouration: Body transparent and with dark yellow patch in the middle of cephalothorax. Eye red or colourless. The male is iridescent appears with dazzling dark blue colour in reflected light.

Salient features: The cephalothorax nearly twice as long as the abdomen (without the furcal rami). Furcal rami longer than the abdomen. The anterior end of cephalothorax quadrangular in shape. The abdominal joints denticulate. The fifth legs are represented by a pair of knob-like projection tipped with a seta each. The male resembles *Sapphirina* in general shape.

Remarks: Richard Hesse states that "the crustacean *Cophilia mirabilia* is confined to temperatures between 23° and 29°" (p. 157, 1937).

Copilia vitrea (Haeckel).

(Karl Lehnhofer, 1926, p. 134-135. Text Fig. 12, 1926).

Occurrence: A single female was present in the townet collection made on 7-1-1948. This is the first record of the occurrence of this copepod at Madras.

Distribution: Pacific, Atlantic, Messina, Indian ocean, Gulf of Guinea, Sargossa sea, Banda sea.

Size: 4·5 mm.

Colour: Transparent and colourless.

Salient Features: The copepod has a very robust appearance. The corneal lens is very prominent. The fourth thoracic segment has a spinous projection in the middle. The sides of the fifth thoracic segment produced acutely. Genital segment indistinctly divided. Antenna long. The fifth leg is represented by a knob-like projection tipped with a stout claw, a short spine and a long spine at the base.

Sub-order: Monstrilloida.

Genus: *Monstrilla* Dana 1848.

Monstrilla sp. (Fig. 17 and 18).

Occurrence: Three females of this copepod were present in the plankton from Kundugal channel collected in March.

Colour: Formalin preserved specimens appear colourless.

Size: Length 1·6 mm.

Salient features: The cephalosome is nearly as long as the thorax and abdomen. The antennule is indistinctly five jointed. The exopodites of the swimming feet longer than the endopodite. The fifth legs represented by a small lamellar process which is cleft in the middle, the inner lobe carrying two and the outer lobe three setae. Furca carrying five setae.

Sub-order: Caligoida.

Family: CALIGIDAE.

Genus: *Caligus* Muller.

Caligus savala Gnanamuthu.

(Gnanamuthu 1948, p. 591, Figs. 1, 2 and 3).

This is found to occur commonly in the plankton from August to March and several males and females have been collected.

Size: Female 3·56 mm. Male 3·38 mm.

Colour: Of a dirty yellowish brown colour.

Salient features: Carapace half as long as the total length of the animal. Lunnules large and shallow. Free thoracic segment very short. Posterior margin of the cephalothorax deeply notched on either side. Genital segment narrow in front, broad behind. Abdomen single jointed, broader than long. Anal laminae bear six plumose setae. Fifth leg represented by three setae. In the male, the genital segment bears two long transverse grooves. Abdomen two jointed. Vestigeal fifth legs visible dorsally.

Caligus sp. (Fig. 19).

Four specimens of this form were present in the plankton collected off Pulli Island in September 1937.

Length: Female 1·8 mm.

Colour: Formalin preserved forms yellow in colour.

Salient features: The abdomen is longer than the carapace. The antennule scarcely visible on the dorsal side. Genital segment very swollen; longer than broad. Lunules prominent. Caudal furca bears four setae. The fifth leg is two jointed, the second joint is long, slender and is tipped with a slender spine.

(To be continued)

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A Non-Parametric Two Sample Test

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ABSTRACT

The sum of squares of the lengths of runs (resulting from the arrangement of the observations of two random samples, each of size n, in ascending order of magnitude) is discussed as a non-parametric two-sample test criterion and the moments of its distribution are derived. A Type VI function has been fitted to this distribution (for $6 \leqslant n \leqslant 15$) after studying the behaviour of the coefficients β_1, β_2 and the Pearsonian criterion k. The levels of significance of the fitted distribution have been computed.

1. INTRODUCTION

Non-parametric criteria have been developed for testing the hypothesis that two random samples arise from the same population, when nothing is known about the parent distribution functions except that they are continuous. Wald and Wolfowitz (1940), Dixon (1940) and Mathisen (1943) have developed different criteria to test this hypothesis. Swed and Eisenhart (1943) used the Wald—Wolfowitz statistic as a test of randomness also and have computed extensive tables for using this criterion. One of the present authors (1951) has improved the Wald—Wolfowitz criterion by taking into account the variation in run patterns and has provided the necessary tables.

In this paper, a new criterion, namely the sum of squares of lengths of runs, is discussed (for samples of the same size). The expressions for the first three moments of this criterion have been derived in full, while for the fourth moment only an asymptotic expression has been obtained.

2. The Test Criterion

The observations of the two samples (each of size n) may be pooled together and arranged in ascending order of magnitude. Denoting by X and Y, a member of the first and second sample respectively, this sequence of observations can be translated into a series of runs of X's and Y's [Ref. Ramachandran (1951) Sec. 2]. It may be seen that this is one of a large number of possible run patterns. If the samples were large and had arisen from the same population, there will be a thorough intermingling of X's and Y's and we could expect the ideal run pattern in which all the runs are of length unity. Due to limitations of sampling, the resulting pattern would be far from the ideal one, but we could reasonably expect the X's and Y's to distribute among themselves "uniformly", thus producing a run pattern without much variation in the lengths of runs. Thus, a pattern having smaller variation in the lengths of runs may be considered more favourable to the null hypothesis. But, if the samples had arisen from different populations, there is likely to be a diminution in the total number of runs. (Wald—Wolfowitz concept). Hence, in forming a two-sample test criterion, the variations in run lengths have to be considered along with the total number of runs.

The sum of squares of lengths of runs, namely,

$$N = \sum_{j=1}^{n} j^2 N_{1j} + \sum_{j=1}^{n} j^2 N_{2j}$$

(where N_{1j} and N_{2j} are the number of X and Y runs respectively, of length j) is a statistic which takes into account both these aspects together. Patterns having a large number of runs and small variations in lengths of runs, give a small value for N, whereas, if the number of runs were small or the variations in lengths were large, we have a larger value for N. It may also be seen that if the number of runs were small and the variations in lengths of runs, large, N takes a still more larger value. For example,

1. XXXX YYY XX Y X YYY	gives	$N = 40$
2. XXXXX YYY X Y X YYY	,,	$N = 46$
3. XXXX YYY XX YYYY X	,,	$N = 46$
4. XXXXX YYY X YYYY X	,,	$N = 52$

Thus, the sum of squares of lengths of runs may reasonably be chosen as a test criterion for the hypothesis that two samples of the same size come from the same population.

3. The Moments of the Distribution of N.

Mood (1940) has derived the following expressions for the generalised factorial moments of the distribution of N_{1j} and N_{2j}.

$$E\left(\prod_j N_{1j}^{(a_j)}\right) = E\left(\prod_j N_{2j}^{(a_j)}\right)$$

$$= \frac{1}{2nC_n}\left[(n+1)^{(\Sigma a_j)} \binom{2n - \Sigma(j+1)a_j}{n - \Sigma j a_j} \right] \qquad .. \quad (1)$$

$$E\left(\prod_{j,k} N_{1j}^{(a_j)} N_{2k}^{(b_k)}\right)$$

$$= \frac{1}{2nC_n}\left[\sum_{N_1, N_2} N_1^{(\Sigma a_j)} N_2^{(\Sigma b_k)} \right.$$

$$\left. \binom{n - \Sigma j a_j - 1}{N_1 - \Sigma a_j - 1}\binom{n - \Sigma k b_k - 1}{N_2 - \Sigma b_k - 1} F(N_1, N_2) \right] \qquad .. \quad (2)$$

where $x^{(p)} = x(x-1) \ \ldots \ (x-p+1)$,

$$N_1 = \sum_{j=1}^{n} N_{1j} ; \; N_2 = \sum_{j=1}^{n} N_{2j},$$

and
$$\begin{aligned} F(N_1, N_2) &= 0 \quad \text{if} \quad |N_1 - N_2| > 1. \\ &= 1 \quad \text{if} \quad |N_1 - N_2| = 1. \\ &= 2 \quad \text{if} \quad |N_1 - N_2| = 0. \end{aligned}$$

In (2) the summation over N_2 is accomplished by putting $N_2 = N_1 - 1$, N_1 and $N_1 + 1$ successively and then, the summation with respect to N_1 is performed for $1 \leqslant N_1 \leqslant n$.

In deriving the expressions for the moments of the distribution of N, we need employ only (1) and (2), since with each run pattern, the values of N_{1j}, N_{2j} alone change for a given value of j. The expressions for the first and second moments given below, were published by the former author in a previous paper (1952). The third moment and the asymptotic form of the fourth moment [sec. 3·3 and 3·4 *] have, however, been obtained by a different method,† which is illustrated in sec. 3·3.

* Sec. 3·3 was derived by the former author and sec. 3·4, by the latter.

† The authors are indebted to Mr. V. J. Chacko, Research Officer, Statistical Laboratory, Trivandrum, for drawing their attention to this method.

3·1. *The First Moment*

$$\mathrm{E}(\mathrm{N}) = \sum_{j=1}^{n} j^2\,\mathrm{E}(\mathrm{N}_{1j}) + \sum_{j=1}^{n} j^2\,\mathrm{E}(\mathrm{N}_{2j})$$

$$= 2\,(n+1)^{(2)} \sum_{j=1}^{n} j^2 \frac{n^{(j)}}{(2n)^{(j+1)}}$$

$$= \frac{6n^2}{(n+2)} \qquad \ldots \quad (3)$$

3·2. *The Second Moment*

$$\mathrm{E}(\mathrm{N}^2) = 2 \sum_{j=1}^{n} j^4\,\mathrm{E}(\mathrm{N}_{1j}^2) + 2 \sum_{j \neq k=1}^{n} j^2k^2\,\mathrm{E}(\mathrm{N}_{1j}\,\mathrm{N}_{1k})$$

$$+ 2 \sum_{j,\,k=1}^{n} j^2k^2\,\mathrm{E}(\mathrm{N}_{1j}\,\mathrm{N}_{2k})$$

$$= 2\,(n+1)^{(2)} \sum_{j=1}^{n} j^4 \frac{n^{(j)}}{(2n)^{(j+1)}}$$

$$+ 2n^{(2)}\,(n+1)^{(2)} \sum_{j,\,k=1}^{n} j^2k^2 \frac{n^{(j+k)}}{(2n)^{(j+k+2)}}$$

$$+ 2 \left\{ 2 \sum_{j,\,k=1}^{n} j^2k^2 \frac{n^{(j)}\,n^{(k)}}{(2n)^{(j+k)}} \right.$$

$$\left. + 4 \sum_{j,\,k=1}^{n} j^2k^2 \frac{n^{(j+1)}\,n^{(k+1)}}{(2n)^{(j+k+1)}} + \sum_{j,\,k=1}^{n} j^2k^2 \frac{n^{(j+2)}\,n^{(k+2)}}{(2n)^{(j+k+2)}} \right\}$$

$$= \frac{4\,(9n^6 + 66n^5 + 69n^4 - 62n^3 - 18n^2 + 200n + 96)}{(n+4)^{(4)}} - \frac{16}{2n\mathrm{C}_n}$$

Hence

$$\mu_2(\mathrm{N}) = \frac{8[6n^6 + 15n^5 - 16n^4 - 71n^3 + 82n^2 + 248n + 96]}{(n+4)^{(4)}\,(n+2)} - \frac{16}{2n\mathrm{C}_n} \qquad \ldots \quad (4)$$

3·3. *The Third Moment*

$$E(N^3) = 2 \sum_{j=1}^{n} j^6 E(N_{1j}^3) + 6 \sum_{j\neq k=1}^{n} j^4k^2 E(N_{1j}^2 N_{1k})$$

$$+ 12 \sum_{j\neq k\neq l=1}^{n} j^2k^2l^2 E(N_{1j} N_{1k} N_{1l})$$

$$+ 6 \sum_{j,\, k=1}^{n} j^4k^2 E(N_{1j}^2 N_{2k}) + 12 \sum_{j\neq k,\, l=1}^{n} j^2k^2l^2 E(N_{1j} N_{1k} N_{2l})$$

$$= 2(n+1)^{(2)} \sum_{j=1}^{n} j^6 \frac{n^{(j)}}{(2n)^{(j+1)}}$$

$$+ 6n^{(2)} (n+1)^{(2)} \sum_{j,\, k=1}^{n} j^4k^2 \frac{n^{(j+k)}}{(2n)^{(j+k+2)}}$$

$$+ 2n^{(3)} (n+1)^{(3)} \sum_{j,\, k,\, l=1}^{n} j^2k^2l^2 \frac{n^{(j+k+l)}}{(2n)^{(j+k+l+3)}}$$

$$+ 6 \left\{ 2 \sum_{j,\, k=1}^{n} j^4k^2 \frac{n^{(j)} n^{(k)}}{(2n)^{(j+k)}} + 4 \sum_{j,\, k=1}^{n} j^4k^2 \frac{n^{(j+1)} n^{(k+1)}}{(2n)^{(j+k+1)}} \right.$$

$$\left. + \sum_{j,\, k=1}^{n} j^4k^2 \frac{n^{(j+2)} n^{(k+2)}}{(2n)^{(j+k+2)}} \right\}$$

$$+ 6 \left\{ \sum_{j,\, k,\, l=1}^{n} j^2k^2l^2 (n-j-k-1)^{(2)} (n-l-1) \frac{n^{(j+k)} n^{(l+3)}}{(2n)^{(j+k+l+3)}} \right.$$

$$+ 2 \sum_{j,\, k,\, l=1}^{n} j^2k^2l^2 (n-j-k-1)^{(2)} \frac{n^{(j+k-1)} n^{(l+3)}}{(2n)^{(j+k+l+2)}}$$

$$+ 6 \sum_{j,\, k,\, l=1}^{n} j^2k^2l^2 (n-j-k-1)(n-l-1) \frac{n^{(j+k)} n^{(l+2)}}{(2n)^{(j+k+l+2)}}$$

$$+ 12 \sum_{j,\, k,\, l=1}^{n} j^2k^2l^2 (n-j-k-1) \frac{n^{(j+k-1)} n^{(l+2)}}{(2n)^{(j+k+l+1)}}$$

$$+ 6 \sum_{j,\, k,\, l=1}^{n} j^2k^2l^2 (n-l-1) \frac{n^{(j+k)} n^{(l+1)}}{(2n)^{(j+k+l+1)}}$$

$$+ 12 \sum_{j,\,k,\,l=1}^{n} j^2k^2l^2 \frac{n^{(j+k-1)}\,n^{(l+1)}}{(2n)^{(j+k+l)}} \Bigg\} \quad \ldots \quad (5)$$

The first term in (5) can be evaluated as follows.

Let $u_j = \dfrac{n^{(j)}}{(2n)^{(j+1)}}$ and $V_j = \dfrac{j^6 n^{(j+1)}}{(2n)^{(j+1)}}$

$$V_j - V_{j-1} = \frac{j^6 n^{(j+1)}}{(2n)^{(j+1)}} - (j-1)^6 \frac{n^{(j)}}{(2n)^{(j)}}$$

$$= u_j \,[j^6(-n-6) + 3j^5(4n+5) - 10j^4(3n+2) + 5j^3(8n+3) - 6j^2(5n+1) + j\,(12n+1) - 2n]$$

Summing both sides $w.r.$ to j, (j running from 1 to n) we have,

$$V_n - V_o = 0$$

$$= \sum_{j=1}^{n} u_j[-j^6(n+6) + 3j^5(4n+5) - 10j^4(3n+2) + 5j^3(8n+3) - 6j^2(5n+1) + j\,(12n+1) - 2n]$$

Hence $\displaystyle\sum_{j=1}^{n} j^6 \frac{n^{(j)}}{(2n)^{(j+1)}}$

$$= \frac{1}{(n+6)} \sum_{j=1}^{n} \frac{n^{(j)}}{(2n)^{(j+1)}} [3j^5(4n+5) - 10j^4(3n+2) + 5j^3(8n+3) - 6j^2(5n+1) + j\,(12n+1) - 2n].$$

Evaluating Σu_j, $\Sigma j u_j$, ..., $\Sigma j^5 u_j$ on similar lines, we have, for the first term in (5),

$$2(n+1)^{(2)} \sum_{j=1}^{n} j^6 \frac{n^{(j)}}{(2n)^{(j+1)}}$$

$$= \frac{42n^3(223n^3 - 126n^2 + 17n + 6)}{(n+6)^{(5)}} \quad \ldots \quad (6)$$

Proceeding on similar lines, it can be easily seen that the remaining terms in (5) give rise to the expressions given below.

$$6n^{(2)}(n+1)^{(2)} \sum_{j,\,k=1}^{n} j^4k^2 \frac{n^{(j+k)}}{(2n)^{(j+k+2)}}$$

$$\doteq \frac{6n^2(n-1)(225n^4-350n^3+327n^2-202n-24)}{(n+6)^{(5)}} \quad \ldots \quad (7)$$

$$2n^{(3)}(n+1)^{(3)} \sum_{j,\,k,\,l=1}^{n} j^2k^2l^2 \frac{n^{(j+k+l)}}{(2n)^{(j+k+l+3)}}$$

$$\doteq \frac{18n^2(n-1)^2(n-2)(3n^3-5n^2+8n-4)}{(n+6)^{(5)}} \quad \ldots \quad (8)$$

The individual terms in the two double brackets lead to very complicated expressions and hence, only the simplified expressions are given. Denoting by A_1, the sum of the terms in the first double bracket and by A_2, the sum of the terms in the second, we have

$$6A_1 = 6\,\frac{(225n^6+362n^5-1957n^4-1374n^3+9856n^2+14608n+4800)}{(n+4)^{(4)}} - \frac{48}{2nC_n}(6n^2+24n+25) \quad \ldots \quad (9)$$

$$6A_2 = 6\,\frac{(27n^7-21n^6-309n^5+757n^4+1610n^3-4576n^2-9104n-3264)}{(n+4)^{(4)}} + \frac{48}{2nC_n}(2n^2+12n+17) \quad \ldots \quad (10)$$

The sum of the expressions (6), (7), (8), (9), and (10) gives

$$E(N^3) = 12\,(18n^9 + 342n^8 + 2198n^7 + 3106n^6 \\ - 5996n^5 - 14112n^4 + 35412n^3 \\ \frac{+ 110264n^2 + 91008n + 23040)}{(n+6)^{(6)}} \\ - \frac{192\,(n+2)\,(n+1)}{2nC_n}$$

Hence,

$$\mu_3(N) = \frac{48}{(n+6)^{(6)}\,(n+2)^2} \begin{array}{l} \{50n^9 - 255n^8 + 37n^7 - 4501n^6 \\ - 8315n^5 + 33014n^4 \\ + 142940n^3 + 198392n^2 \\ + 114048n + 23040\} \end{array}$$

$$- \frac{96}{(n+2)\,.2nC_n}\,(2n^3 + 7n^2 + 16n + 8) \qquad \ldots \quad (11)$$

For large values of n, since the second term in (11) is negligible, the coefficient $\beta_1 (= \mu_3^2/\mu_2^3)$ is given by

$$\frac{52\cdot07}{n} - \frac{588\cdot5}{n^2} + O\,(\frac{1}{n^3}) \qquad \ldots \quad (12)$$

3·4. *The Asymptotic Expression for the Fourth Moment*

$$E(N^4) = 2 \sum_{j=1}^{n} j^8\,E(N_{1j}^4) + 8 \sum_{j\neq k=1}^{n} j^6k^2\,E(N_{1j}^3\,N_{1k})$$

$$+ 12 \sum_{j\neq k=1}^{n} j^4k^4\,E(N_{1j}^2\,N_{1k}^2)$$

$$+ 24 \sum_{j\neq k\neq l=1}^{n} j^4k^2l^2\,E(N_{1j}^2\,N_{1k}\,N_{1l})$$

$$+ 48 \sum_{j\neq k\neq l\neq m=1}^{n} j^2k^2l^2m^2\,E(N_{1j}\,N_{1k}\,N_{1l}\,N_{1m})$$

$$+ \;8 \sum_{j,\,k=1}^{n} j^6k^2\,E(N_{1j}^3\,N_{2k})$$

$$+ 24 \sum_{j\neq k,\,l=1}^{n} j^4k^2l^2\,E(N_{1j}^2\,N_{1k}\,N_{2l})$$

$$+ 48 \sum_{j \neq k \neq l,\, m=1}^{n} j^2k^2l^2m^2 \, E(N_{1j} N_{1k} N_{1l} N_{2m})$$

$$+ 6 \sum_{j,\, k=1}^{n} j^4k^4 \, E(N_{1j}^2 N_{2k}^2)$$

$$+ 24 \sum_{j \neq k,\, l=1}^{n} j^2k^2l^4 \, E(N_{1j} N_{1k} N_{2l}^2)$$

$$+ 24 \sum_{j \neq k,\, l \neq m=1}^{n} j^2k^2l^2m^2 \, E(N_{1j} N_{1k} N_{2l} N_{2m}) \qquad \ldots \quad (13)$$

Substituting the expressions for $E(N_{1j}^4)$, $E(N_{1j}^3 N_{1k}), \ldots \ldots$ $\ldots\ldots$ $E(N_{1j}^3 N_{2k})$ .... etc., from (1) and (2), and retaining only those terms* which will give rise to n^2 and higher powers of n (after summation), the expression (13) reduces to

$$2n^{(4)}(n+1)^{(4)} \sum j^2k^2l^2m^2 \frac{n^{(j+k+l+m)}}{(2n)^{(j+k+l+m+4)}}$$

$$+ 12n^{(3)}(n+1)^{(3)} \sum j^4k^2l^2 \frac{n^{(j+k+l)}}{(2n)^{(j+k+l+3)}}$$

$$+ 8n^{(2)}(n+1)^{(2)} \sum j^6k^2 \frac{n^{(j+k)}}{(2n)^{(j+k+2)}}$$

$$+ 6n^{(2)}(n+1)^{(2)} \sum j^4k^4 \frac{n^{(j+k)}}{(2n)^{(j+k+2)}}$$

$$+ 288 \sum (n-j-k-l-1)(n-m-1) j^2k^2l^2m^2 \frac{n^{(j+k+l-1)} n^{(m+3)}}{(2n)^{(j+k+l+m+2)}}$$

$$+ 96 \sum (n-j-k-l-1)^{(2)} (n-m-1) j^2k^2l^2m^2 \frac{n^{(j+k+l-1)} n^{(m+4)}}{(2n)^{(j+k+l+m+3)}}$$

$$+ 8 \sum (n-j-k-l-1)^{(3)} (n-m-1) j^2k^2l^2m^2 \frac{n^{(j+k+l-1)} n^{(m+5)}}{(2n)^{(j+k+l+m+4)}}$$

$$+ 192 \sum (n-j-k-l-1)^{(2)} j^2k^2l^2m^2 \frac{n^{(j+k+l-2)} n^{(m+4)}}{(2n)^{(j+k+l+m+2)}}$$

$$+ 16 \sum (n-j-k-l-1)^{(3)} j^2k^2l^2m^2 \frac{n^{(j+k+l-2)} n^{(m+5)}}{(2n)^{(j+k+l+m+3)}}$$

* The summations for these terms are for $1 \leqslant j, k, l, m \leqslant n$.

$$+ 24 \Sigma \, (n-j-k-1)^{(2)} (n-l-1) j^4k^2l^2 \frac{n^{(j+k)} n^{(l+3)}}{(2n)^{(j+k+l+3)}}$$

$$+ 144 \Sigma \, (n-j-k-1) (n-l-1) j^4k^2l^2 \frac{n^{(j+k)} n^{(l+2)}}{(2n)^{(j+k+l+2)}}$$

$$+ 48 \Sigma \, (n-j-k-1)^{(2)} j^4k^2l^2 \frac{n^{(j+k-1)} n^{(l+3)}}{(2n)^{(j+k+l+2)}}$$

$$+ 8 \Sigma \, j^6k^2 \frac{n^{(j+2)} n^{(k+2)}}{(2n)^{(j+k+2)}}$$

$$+ 6 \Sigma \, (n-j-k-1)^{(2)} (n-l-m-1)^{(2)} j^2k^2l^2m^2 \frac{n^{(j+k+2)} n^{(l+m+2)}}{(2n)^{(j+k+l+m+4)}}$$

$$+ 36 \Sigma \, (n-j-k-1)^{(2)} (n-l-m-1) j^2k^2l^2m^2 \frac{n^{(j+k+1)} n^{(l+m+2)}}{(2n)^{(j+k+l+m+3)}}$$

$$+ 36 \Sigma \, (n-j-k-1)^{(2)} j^2k^2l^2m^2 \frac{n^{(j+k)} n^{(l+m+2)}}{(2n)^{(j+k+l+m+2)}}$$

$$+ 36 \Sigma \, (n-j-k-1) (n-l-m-1)^{(2)} j^2k^2l^2m^2 \frac{n^{(j+k+2)} n^{(l+m+1)}}{(2n)^{(j+k+l+m+3)}}$$

$$+ 216 \Sigma \, (n-j-k-1) (n-l-m-1) j^2k^2l^2m^2 \frac{n^{(j+k+1)} n^{(l+m+1)}}{(2n)^{(j+k+l+m+2)}}$$

$$+ 36 \Sigma \, (n-l-m-1)^{(2)} j^2k^2l^2m^2 \frac{n^{(j+k+2)} n^{(l+m)}}{(2n)^{(j+k+l+m+2)}}$$

$$+ 12 \Sigma \, (n-j-k-1)^{(2)} (n-l-1) j^2k^2l^4 \frac{n^{(j+k)} n^{(l+3)}}{(2n)^{(j+k+l+3)}}$$

$$+ 24 \Sigma \, (n-j-k-1)^{(2)} j^2k^2l^4 \frac{n^{(j+k-1)} n^{(l+3)}}{(2n)^{(j+k+l+2)}}$$

$$+ 72 \Sigma \, (n-j-k-1) (n-l-m-1) j^2k^2l^4 \frac{n^{(j+k)} n^{(l+2)}}{(2n)^{(j+k+l+2)}}$$

$$+ 6 \Sigma \, j^4k^4 \frac{n^{(j+2)} n^{(k+2)}}{(2n)^{(j+k+2)}}$$

The above terms are calculated by the method suggested in 3·3 and the results (keeping only n^2 and higher powers of n) are summarised in the following table:

Terms in Serial Order	Coefficient of n^4	Coefficient of n^3	Coefficient of n^2
1	162	— 7,668	197,166
2	—	8,100	— 343,980
3	—	—	112,392
4	—	—	33,750
5	—	—	93,312
6	—	15,552	— 612,576
7	648	— 34,344	763,464
8	—	—	62,208
9	—	2,592	— 122,832
10	—	16,200	— 597,096
11	—	—	194,400
12	—	—	64,800
13	—	—	112,392
14	486	— 20,196	370,194
15	—	5,832	— 183,060
16	—	—	11,664
17	—	5,832	— 183,060
18	—	—	69,984
19	—	—	11,664
20	—	8,100	— 240,156
21	—	—	32,400
22	—	—	97,200
23	—	—	33,750
Total ..	1296	—	— 22,020

Hence

$$E(N^4) \simeq 1296n^4 - 22020n^2$$

and $$\mu_4(N) \simeq 8508n^2 \qquad .. \quad (14)$$

The asymptotic value of β_2 is given by

$$\beta_2 = \frac{\mu_4(N)}{\mu_2^2(N)} \simeq \frac{8508n^2}{48 \times 48n^2}$$
$$= 3\cdot69 \qquad .. \quad (15)$$

4. THE BEHAVIOUR OF β_1 AND β_2

For values of n greater than 8, it is very difficult to write down the frequency distribution of N. But a study of the distribution for small values of n [ref. Ramachandran (1952), Sec. 3] indicates that it can be approximated, for testing purposes by a continuous curve.

In a previous paper (1952), the former author had made a conjecture that β_1 and β_2 steadily increase with n and appear to be tending to some definite values. But the expressions for β_1 and the asymptotic form of β_2 [given in Sec. 3 (12) and (15)] suggest that both of them attain a maximum value and begin to decrease after a certain stage. The values of these coefficients were calculated for the distribution of N for $n = 12$ (given in the appendix), in order to study the trend of β_1 and β_2. These values along with those for $n = 6$, 7 and 8 seem to indicate that β_1 and β_2 both attain a maximum for some value of n between 8 and 12.

The values of the Pearsonian criterion k (given in Table I) are found to be steadily decreasing with n and since the asymptotic value of β_1 is $O\left(\frac{1}{n}\right)$ and of β_2 is 3·7, there is reason to believe that the fitted distribution will undergo a transition from Type VI to Types V and IV and finally to Type VII when n is very large. The exact transition stage cannot be determined unless the expression for $\mu_4(N)$ and hence β_2 is studied in more detail. (This study is being taken up by the second author and it is hoped to publish the results at a later date).

TABLE I

n	β_1	β_2	k
6	1·834	5·859	10·450
7	1·976	6·322	3·089
8	2·041	6·600	2·150
12	1·974	6·798	1·331

The trend of β_2 indicates that k will be greater than unity* for values of $n \leqslant 15$, and hence it is safe to fit a Type VI function

* Since the exact value of β_1 is known for $n = 15$, it can be seen that $k = \dfrac{\beta_1(\beta_2+3)^2}{4(4\beta_2-3\beta_1)(2\beta_2-3\beta_1-6)}$ will be greater than unity if β_2 lies between 0·99 and 6·76.

for the distribution of N (for $6 \leqslant n \leqslant 15$). In order to facilitate the use of the statistic N to test the hypothesis that two random samples (of the same size) come from the same population, the various levels of significance (of the true distribution for $3 \leqslant n \leqslant 5$, and of the fitted one for $6 \leqslant n \leqslant 15$) have been calculated and presented in Table III. (Since N takes only even values, the actual level of significance calculated from the fitted function has been corrected to the next highest even integer.) Table II affords a comparative study of these levels, for certain values of n, for the true and fitted distributions.

TABLE II

n	10% level		1% level	
	True distribution	Fitted distribution	True distribution	Fitted distribution
6	40	38	58	58
8	54	54	78	80
12	86	84	118	118

TABLE III

Values of N′ such that $P[N \geqslant N'] \leqslant \alpha$

$n \backslash \alpha$	0·10	0·05	0·025	0·01
3	18	18	18	18
4	26	32	32	32
5	34	38	42	50
6	38	44	50	58
7	46	52	60	68
8	54	60	68	80
9	62	70	78	90
10	68	78	86	100
11	76	86	96	108
12	84	94	104	118
13	92	102	112	128
14	98	110	122	136
15	106	118	130	146

5. Remarks

Even though it has been assumed that the distributions are continuous, practical situations may arise wherein two sample observations are identical. In such cases, it is suggested that the run pattern giving the larger value for N, be used for reference to Table III.

The use of the statistic N as a two-sample test criterion, is illustrated by the following examples.

(1) $n = 7$

Sample I. 82, 50, 39, 22, 57, 32, 96.

Sample II. 101, 119, 117, 104, 99, 68, 118.

Combining the two samples and arranging the observations in ascending order of magnitude, we have the following sequence in X's and Y's

XXXXX Y XX YYYYYY.

$N = 5^2 + 1^2 + 2^2 + 6^2 = 66.$

From Table III, $0 \cdot 01 < P[N \geqslant 66] < 0 \cdot 025$. Hence, the hypothesis that the two samples are from the same population, is to be rejected at 2·5% level, but can be accepted at 1% level.

(2) $n = 10$.

Sample I. 1·25, 1·50, 2·00, 2·00, 2·25, 2·75, 2·75, 2·75, 3·25, 3·25.

Sample II. 1·50, 1·50, 1·75, 1·75, 2·00, 2·00, 2·00, 2·50, 2·50, 3·00.

It may be seen by arranging the 20 observations in ascending order of magnitude that the pattern

XX YYYYYY XXX YY XXX Y XX

gives the maximum value of 80 for N. Referring to Table III

$$0 \cdot 025 < P[N \geqslant 80] < 0 \cdot 05$$

Hence the hypothesis is to be rejected at 5% level and to be accepted at 2·5% level.

In conclusion, the authors would like to express their grateful thanks to Dr. P. B. Patnaik, for his helpful suggestions.

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APPENDIX

Frequency Distribution of N for $n = 12$

N	Frequency	N	Frequency	N	Frequency
24	2	84	38952	144	674
26	22	86	29892	146	464
28	242	88	27724	148	536
30	990	90	21420	150	84
32	4270	92	22246	152	472
34	9360	94	17716	154	292
36	22472	96	15642	156	336
38	37392	98	10336	158	228
40	59282	100	12912	160	272
42	82080	102	9468	162	92
44	109118	104	10080	164	184
46	120302	106	6868	166	72
48	149600	108	6786	168	104
50	151474	110	4932	170	92
52	163024	112	5184	172	168
54	157188	114	3752	174	42
56	164064	116	4792	176	152
58	146398	118	2640	178	64
60	150512	120	2802	180	8
62	128702	122	1808	182	12
64	126064	124	2764	184	64
66	104258	126	1572	186	24
68	107192	128	2024	188	96
70	83600	130	1608	190	24
72	83188	132	1096	192	12
74	66840	134	840	194	36
76	65680	136	1082	196	40
78	50058	138	526	198 — 288	154
80	48552	140	1048		
82	38428	142	492	Total ..	2704156

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Aromatisation and Isomerisation of Bicyclic Terpenes

BY

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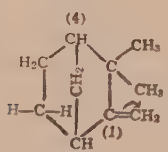
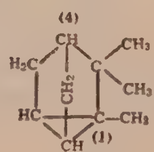
Fujita[1] (1932) reported the conversion of camphene (I) to p-cymene (V) (represented in the nonplanar form for context emphasis) by heating with phosphorus pentoxide and sulphur, but apparently offered no explanation. The following is now submitted as a sequence of legitimate stages leading to the formation of p-cymene.





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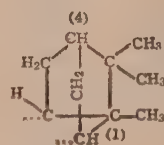

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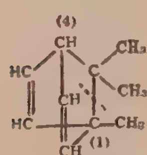

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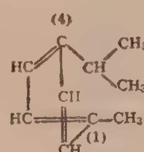
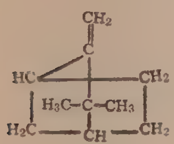


V

The first stage is the formation of the tricyclene (II), then breaking of one of the cyclopropane bonds to give (III). At this stage partial aromatisation can take place to a compound of the type (IV) which breaks along the dotted line to yield p-cymene (V).

The same author obtained a number of products by heating camphene (I) with 89% phosphoric acid at 200° for 10 hours. To one of the products he has assigned a constitution based on the isolation of the cyclopropane dicarboxylic acid (XIX) in oxidative degradation. It is now suggested that a compound (XI), isomeric with thujene, may be one of the other unidentified products.

Ipatiev and Pines[4] (1945) reported the formation of 1-ethyl-2-isopropylcyclopentene (XII) from pinene. These interesting changes become intelligible along the following lines. Camphene (VI)



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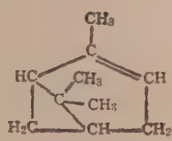

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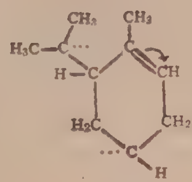

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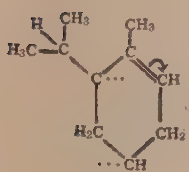

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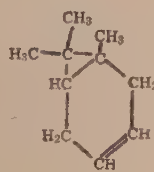
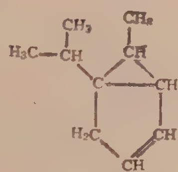
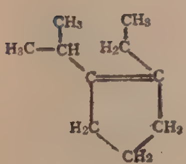


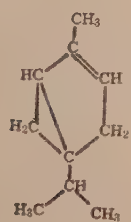
X



XI



XII



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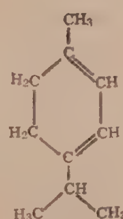

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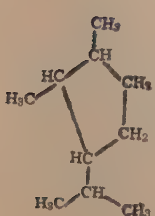

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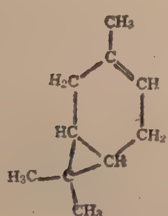

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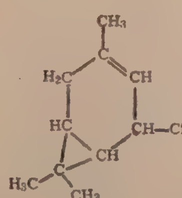


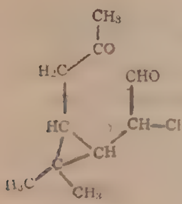
XVII

isomerises to pinene (VII). Pinene obeys the Schmidt rule[8] (1935) which explains the mechanism of pyrolysis of several organic compounds on the theory that the presence of double bonds reinforces the single bonds which are separated from the double bond by an odd number of carbon atoms and weakens others.

Camphene (VI) isomerises to pinene (VII) and this is followed by the breaking of one of the cyclobutane bonds leading to (VIII) or (IX). It can then isomerise to the carene isomer (X) or the thujene isomer (XI). On page 116 of 'Isomerisation of pure hydrocarbons' (A.C.S. monograph)[3] (1942), it is stated that the survival of the fused cyclopropane ring under the experimental conditions is doubtful. The present author has seen the paper only in abstract and feels the criticism of the American authors is contrary to the experimental report that the cyclopropane dicarboxylic acid (XIX) has been obtained in oxidative degradation. The formation of o-, and p-cymenes from pinane, reported by Olberg, Pines and Ipatiev[7] (1948) presents no difficulty as the first stage of the reaction may be the insipid formation of a monoene or a diene, and is thus another example of the operation of Schmidt rule. The rupture of the bond between methyl and isopropyl of (XI) followed by the formation of a double bond leads to 1-ethyl-2-isopropylcyclopentene (XII), compound (VIII) or (IX) can yield o-cymene, the formation of p-cymene being normal. Thujene (XIII) has been shown by Simonsen[10] (1922) to racemize very slowly on standing and this may be associated with decyclization-isomerisation. Decyclization may be associated with the conversion of the cyclopropane ring into a true double bond in conjugate position (gama-terpinene may be an intermediate, forming another quinonoid system), yielding alpha-terpinene (XIV). Gascoigne[2] (1941) subjected Thujene to the severe action of alcoholic hydrochloric acid under reflux for 15 minutes, followed by a special treatment of the recovered hydrocarbons with maleic anhydride in acetone, yielding 45% of free gama-terpinen-, and 20% of alpha-terpinen-maleic anhydride adduct. The formation of 1:2-dimethyl-3-isopropylcyclopentane (XV) reported by Kasansky[6] (1929) is probably another example of the operation of the Schmidt rule, the cyclopropane bond being affected prior to the saturation of the original double bond. Iyer and Simonsen[5] (1922) found that catalytic hydrogenation of d-carone (XXI) in the presence of colloidal palladium led to the rupture of the cyclopropane ring giving a mixture of p-menthan-2-ol (XXII), p-menthane (XXIII).

It is very well known that a cyclopropane ring approximates very closely to a double bond and so 3-carene (XVI) can be considered to function as a p-quinone and 4-carene (XXIV) as an o-quinone. The aromatisation of 3-carene is the result of the expulsion of two protons from the two p-methylene groups followed

by rearrangement to p-, or m-cymene as demanded by Schmidt rule.



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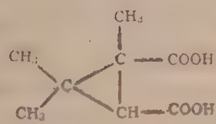

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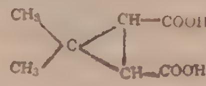

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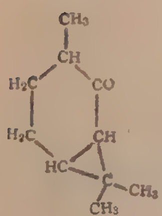

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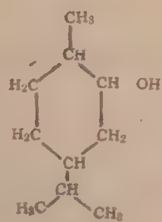

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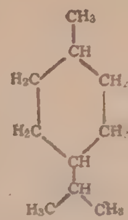

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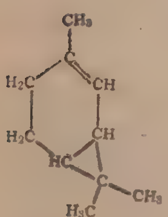

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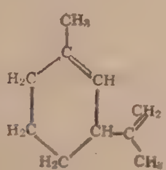
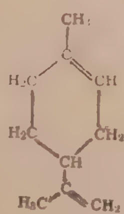


XXV



XXVI

On the basis of the quinonoid conception, the results obtained by Tishchenko and Khovanskay[11] (1950) by chlorination of 3-carene (XVI), the production of an allylic-chloro-derivative, can be understood without assuming, as has been done, the intermediate formation of 3-chloro-4-carene. One methylene carbon atom undergoes mono-chlorination to (XVII) which gives the chloro-keto-aldehyde (XVIII) on ozonolysis, further degraded by oxidation to caronic acid (XX). The isomerisation of 4-carene by heating at 280° for four hours, reported by Semmler and Schiller[9] (1927), yielding sylvestrene (XXV) and alpha-terpinene (XIV) are examples in which the cyclopropane ring breaks in two directions. The formation of sylvestrene is similar to the formation of dipentene from pinene noted by Goldblatt and Palkin[3] (1942). It is difficult to predict the direction in which the bond would break, but it involves

the familiar phenomena of three-carbon isomerisation initiated by Schmidt rule.

The present discussion is confined to isomerisation by decyclisation as a preliminary to aromatisation. One of the simplest form of isomerisation by aromatisation is the formation of alkyl benzenes from alkyl-cyclo-2: 6-hexadiens. These hexadiens are, in many respects, o-quinones and their aromatisation is brought about by the expulsion of two protons. It is instructive to remember that 2:5-diens have to isomerize to 2:6-diens before aromatization can take place.

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A Critical Review on Novolak Syntans

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ABSTRACT

Novolak syntans are sulphonated phenol aldehyde condensation products. Their tanning property is influenced by a number of factors such as the conditions of preparation, presence of the free starting materials, extent of sulphonation, presence of buffer salts and wetting agents in the tanning bath, and also the type of phenol used. It is highly essential that an agreement on the systematic analysis of these syntans is reached at as early a date as possible to expedite further work.

INTRODUCTION

Syntans are synthetic tanning materials that are defined by Chen (1950) as "any synthetic high molecular organic compound or mixture of such compounds capable of converting animal skin into leather". An old belief that syntans are essentially sulphonic acids has to be corrected in the light of modern research which has proved that syntans can be prepared without sulphonic group. Nevertheless, the novolak syntans which are reviewed herein are only sulphonated compounds.

The admitted usefulness of such sulphonated novolaks in the tannery is not only due to their tanning action, but also due to their other functions such as regulation of plumpness, improvement of colour, solvent action on insoluble matter of vegetable tanning materials, germicidal action (Raymond 1925) and above all, their utilisation as combination tanstuffs both with chrome and with vegetable tanning materials. According to Wolesensky (1926) the special value of sulphonated novolaks is however due to their use as "Exchange Tanstuffs" in the place of the vegetable tanning materials.

Formation of Novolaks:

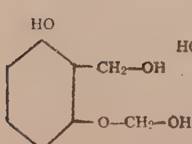
The novolaks are condensation products of phenols with formaldehyde, under the influence of certain catalysts. Ellis (1948) states that they do not harden upon heating to an insoluble, infusible condition but remain soluble and fusible which differentiate them from other resins from the same starting materials. Küntzal and Schwank (1940) have shown that they are formed as the first stage by the acid condensation of phenol and formaldehyde which with longer duration of condensation result in insoluble 'resite' type which are high molecular weight restins.

Experiments by Minoru Imoto and Hiroshi Kakiuchi (1948) amount to prove that the rate of building up of the insoluble resin molecules increases with time of condensation linearly, probably by a simultaneous breaking of the molecules of the soluble portion of the resin. For a given concentration of the catalyst, the equilibrium between the molecular weights of the soluble and the insoluble portions has been found to be fixed. The resin condensation is considered to be an addition-condensation reaction. In acid media the addition process is slower than condensation and the reverse is the case in alkaline media.

The reactions can be represented in the following ways:

$C_6H_5OH + CH_2O \rightarrow HO\text{-}C_6H_4\text{-}CH_2OH$ (Addition Reaction)
$HO\text{-}C_6H_4\text{-}CH_2\text{-}OH + C_6H_5OH \rightarrow HO\text{-}C_6H_4\text{-}CH_2\text{-}C_6H_4\text{-}OH + H_2O$ (Condensation Reaction).

According to Ullmann and Brittner (1909) the initial compound prepared has got the structure (1) whereas F. Oschmann (1947) thinks that the initial compound formed has a structure (2) which is subsequently joined by $\text{-}O\text{-}CH_2OH$ groups. The structure of Oschmann is supported by absorption values. In alkaline medium the compound is considered by Hough (1949) to be (3).



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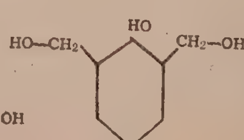
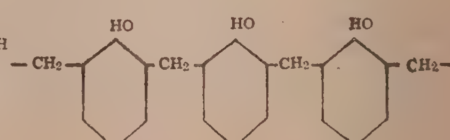


(1)



(3)

Work done by Binko and Kolar (1949), Binko, Lysy and Urbanek (1948) in this connection has shown that novolaks formed by condensing one mole of phenol with more than ·75 moles of formaldehyde but less than ·9 moles can only be used for sulphonation and as tanning agents. According to Wolesensky (1925) and Horn and Komori (1927) higher aldehydes like acetaldehyde and higher phenols like xylenols and resorcinol have given products which have better filling and tanning properties. The use of higher phenols has been patented by Noerr, Mauthe and Doser in 1933.

Sulphonation of Novolaks:

Küntzel (1929) has done sulphonation with sulphuric acid, sodium sulphite and bisulphite. The former gives the sulphonic acid group in the nucleus of the phenol ring whereas the latter in the side chain. A ratio of 2:1 of phenol to sulphuric acid has been found to yield a soluble product which shows a proportion of one sulphonic acid group to every two phenols. Higher phenols require higher quantities of sulphuric acid to make them soluble. The ratio of phenol to sulphuric acid in soluble sulphonated novolaks can be altered if the free unreacted phenol is removed from the reaction system.

Effect of free reactants on the tanning action of sulphonated novolaks:

Free unreacted phenol causes a peptising action on the hide powder as evinced by the work of Wolesensky (1926), Highberger (1936), Balfe and Wallis (1946) and Hough (1949). This effects a solubilising action on the hide protein and causes destruction of the hide, this amount of destruction depending upon the amount of phenol present free in the final product, which in turn depends on the conditions of preparation of the novolak.

As observed by Hough (1949), Gerngross and Bach (1922) and Wilson (1929), free formaldehyde shifts the isoelectric point of collegen, thereby reducing the tanning power of the syntan to a minimum at a pH of about 4. (Hough 1949, graph 1).

Free sulphuric acid has been found by Kohn, Breedis and Crede (1923) and Hough (1949) to have the most deleterious effect in tanning in as much as it might give rise to "acid rot" of leather. Hitherto it has evaded all accepted methods of accurate estimation. However, complete neutralisation diminishes the tanning value of

the syntan as the alkali salts formed are only adsorption tannins as classified by Kohn *et al* (1923).

Effect of condensation and sulphonation on tanning properties of Sulphonated novolaks:

During our investigation on tanning properties vs. time of condensation, it was found that there was an optimum time of condensation for getting the best tanning effects. The same result has also been reported by other authors like Biedermann (1949) and Hough (1949). His graph 2 clearly illustrates that tannins absorbed increases with time of condensation. A similar graph is reported by Biedermann (1949) and observed by us to reach a maximum and then to fall. It may be assumed that particle size of the sulphonated novolak has got a bearing on the tanning properties. It has been assumed and proved latter by Minoru Imoto (1948) *et al* that the particle size increases linearly with time of condensation. Tanning is effected by the penetration and filling of the leather by these particles. Smaller the particles, greater the penetration but lesser the filling action. When the particle size exceeds a certain maximum it becomes too big to penetrate. Then the tanning value of the solution falls. This clearly shows that there should be an optimum particle size which probably corresponds to an optimum time of condensation in order to effect good penetration as well as satisfactory filling action.

Another attempt has been made to explain this peak in the curve on the basis of the same particle size. It was postulated that pure tannins are small particles that can penetrate and combine with hide whereas semi-tannins are larger and deposit on the surface.

To verify this theory novolaks of different sizes have been prepared by Küntzel and Bosse (1947-48) and their tanning power tested. The particle size before sulphonation alone was taken into consideration and it was assumed that sulphonation does not alter particle size. His investigations appear to disprove the above postulate. However, the results of the author have to be taken with reserve as they make without sufficient experimental evidence an assumption that sulphonation does not alter the particle size.

The purity curve (concentration vs. absorbable tannin matter) was plotted by Küntzel (1940) *et al* and was found that it has got a peak similar to the curve obtained earlier by plotting time of condensation with tannin absorption. Still, it will remain

a surmise till experimental evidence is forthcoming that the optimum time of condensation which may correspond to the optimum particle size, corresponds to the optimum concentration in Kuntzel's curve. It is in these lines at present work is done in this Laboratory.

Next to the degree of condensation the degree of sulphonation is the main factor controlling the tanning action. It has been observed that the tanning properties are best when the sulphonation is done to the least extent. The special property of these novolaks as contrasted with other syntans is its optimum solubility, which is also the case with the vegetable tanning materials. Whereas other syntans differ from the vegetable tanning materials in this respect, sulphonated novolaks interestingly enough approach the vegetable tannins and are therefore capable of giving a full leather just like vegetable tanned leather. This property is made possible as it is possible to control the degree of solubility of the novolaks by controlling the degree to which they are sulphonated.

The free sulphonic acids in a sulphonated novolak has been found by Moeller (1920) and Vittorio (1924) to cause a hydrolytic action on the collegen fibres and split the miccellor groups of the hide fibres into free miccels, break down the miccels into free protein molecules, hydrolyse the protein, combine with the hydrolytic products forming free amino acids which form easily soluble condensation products. To prevent this destruction of the hide substance the sulphonated novolaks are neutralised to a certain extent.

It was observed by Küntzel and Grunewald (1947 & 1948) that sulphonated novolaks contain different constituents of different degrees of sulphonation. Constituents of high percentage of sulphonic acid group are less astringent due to their stronger hydrophilic nature than those with less percentage of sulphonic acid group and hence called semi-tannins. This has been proved by the preparation of condensation products that contained no sulphonic acid groups and hence no semi-tannins and possessed excellent tanning properties. Such water soluble novolaks were prepared from resorcinol and formaldehyde.

Sulphonated novolaks precipitate gelatin and if sulphonated only to a small extent are dispersible only in high concentrations. Solubility of a novolak increases with the percentage of sulphonic group and those which contain only less of sulphonic groups are dispersible and remain in suspension whereas those without any

sulphonic group can be dispersed in the presence of certain surface active agents that contain sulphonic acid group like Benzene sulphonic acid, Beta naphthalenesulphonic acid and anthraquinone 2-sulphonic acid.

Other factors influencing the tanning action of Novolaks:

(a) *Influence of Buffering salts*: It was observed by Küntzel *et al* (1950) and Hough (1949) that the percentage of tannins taken from a soluble resin increases greatly in the presence of an organic salt like sodium acetate. A constant pH as obtained with the addition of sodium acetate buffer has been found to be highly desirable during the tanning experiments with novolak syntans. This can be explained either due to a steady supply of the sulphonic acid under the condition of controlled pH by the buffer salt, or due to the peptising action of the undissociated acetic acid (generated from the added sodium acetate) or due to both. To understand this phenomenon, the tanning was done in the presence of urea that has got neither a peptising action nor a buffering action. There was no alteration in the purity curve. When ammonium thiocyanate, which is a powerful peptising agent was used in the place of sodium acetate the purity curve was altered. Vegetable tannins contain fractions consisting of phenolic non-tannins and tannins of low molecular weight that have got a peptising action, similar to that of sodium acetate. The removal by washing of these peptising agents raises the shrinkage temperature of the leather but lowers leather quality. However modifications of the purity curve to resemble those of vegetable tannins does not always indicate an improvement in the tanning property. Tanning heavy hides with the addition of ammonium thiocyanate, produced cracky leather. This can be explained as due to the fact that ammonium thiocyanate has got too great a peptising action on hide substance, and dissolves away the constituent proteins.

(b) *Influence of Non-Tannins:* Non-tannins have been found to act as tanning aids for the regular tanning materials. Synthetic novolaks suffer from want of good penetrating power due to the absence of non-tannins. But this can be rectified by the addition of certain organic acids to the syntan like citric acid and lactic acid after neutralisation.

Influence of nuclear substitution groups on the tanning property of novolaks:

Apart from the solubilising sulphonic acid group in the phenol ring and the condensing $-CH_2OH$ group, the phenols contain other

groups in the nucleus or side chain that have a direct or indirect influence on their tanning properties.

Two such groups are the phenolic OH group that has got a direct bearing on the tanning property and the methyl group in the case of cresols and xylenols that has got an indirect influence on the tanning property.

Conductometric titrations show that the sulphonated novolaks of phenol, meta cresol and ortho cresol contain varying amount of the total titratable, free, pre-existant OH groups, constituting the acidity of the phenols employed. Phenolic OH plays an increasingly important role when its acidity is raised by constitutional changes. It may be recalled that in the case of auxiliary syntans, the phenolic OH is comparatively of lesser significance than in the sulphonated novolaks which are exchange syntans. Phenolic OH is a main group contributing to the tanning action of the vegetable tanning materials.

Other prominent group is methyl group which is present to varying extents in the novolaks prepared from the three cresols and xylenols and also is differently located with respect to the OH group of the phenol used. It was found by Muller and Muller (1948) that in the condensation of phenol with formaldehyde, heat of reaction and the velocity of reaction varied markedly for the first three phenols, and their condensation products differed in their properties. The decreasing order of reactivity of the phenol is 1: 3: 5 xylenol, m-cresol, Phenol, O-cresol, and P-cresol. The progressive substitution of the phenol molecule causes a decrease in their solubility. The formation of a chain like molecules favours associate ions, and thus increases insolubility. These observations are in tune with the observations made by Küntzel (1929) that the novolaks prepared from cresols required a higher amount of sulphuric acid to make them soluble than those from phenol.

As patented by Becherer (1934) the introduction of amino groups into the sulphonated phenol formaldehyde resins has evidently improved their tanning properties. Urea, thiourea, polysulphides, calcium thiocyanate and ammonium thiocyanate have been used in this manner.

The forgoing discussion proves beyond any doubt that the tanning action of a sulphonated novolak is a function not only of the resin as a chemical individual, nor the size, acid or phenolic

character, and the nature of the substituent groups but rather upon all the materials present in the syntan or added to them. While the nature of the novolak resin depends upon the method of preparation and the nature of the raw materials, the success of the tanning depends on the addition to the tanning agent acids, buffers, wetting agents, and other materials. (Küntzel 1950).

Conclusion

The need of the hour is a completely reliable and standardised method of evaluation of the syntans. The nature and constitution of the syntans is comparatively simpler than these of vegetable tanning materials. In spite of this, the progress of work done in this direction to evolve the proper analytical techniques is very poor in comparison with that in the field of vegetable tannins. In 1922, Kohn *et al* stated "It will suffice for the present to prove how different the properties of syntans are. We cannot think of devising generally applicable methods of quantitative determination before we have agreed upon what properties of the constituents of the synthetic tanning materials must be considered, used, and analysed as tanning materials." More than thirty years have passed since this statement and still, no definite conclusions have been arrived at about the evaluation of syntans. Once an agreement is reached on this, the future work in the field can be based on perfectly safe footing.

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